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Fig. 1 shows isolines for the dates of the beginning of the vegetative season for the Prairie Provinces and the major portion of the Northwest Territories. Most of the Arctic Archipelago is omitted because of either the inadequacy of the temperature data or the locations of the stations leading to temperatures

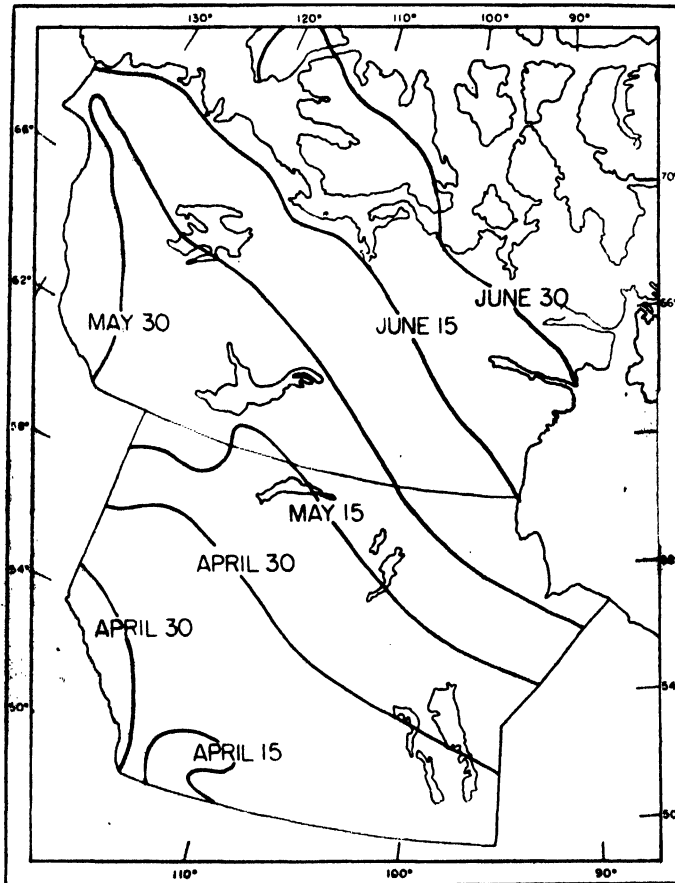


FIG. 1. Isolines for the mean dates of the beginning of the vegetative season for the Prairie Provinces and the Northwest Territories.

that are not typical of the surrounding regions. The isolines are drawn for periods of 15 days, a period commensurate with the representativeness of the temperature data and the scale of the map.

On the average, mean daily temperatures of  $42^{\circ}$  make their first appearance in southern Alberta, reaching Lethbridge and Medicine Hat by Apr. 15, and cross the cultivated region during the next 15 days. The advance northward is more rapid in Alberta than in Saskatchewan and Manitoba. This pattern for the advance is maintained until the Arctic coast between the delta of the Mackenzie River and Coronation Gulf is reached about two months after the start in southern Alberta. The retardation of two to four weeks in

the beginning of the vegetative season in northern Manitoba and the eastern part of the Northwest Territories, compared with the corresponding latitudes along the drainage basin of the Mackenzie, is due mostly to invasions of cold-air masses from off the ice-laden waters of the Arctic Archipelago and Hudson Bay.

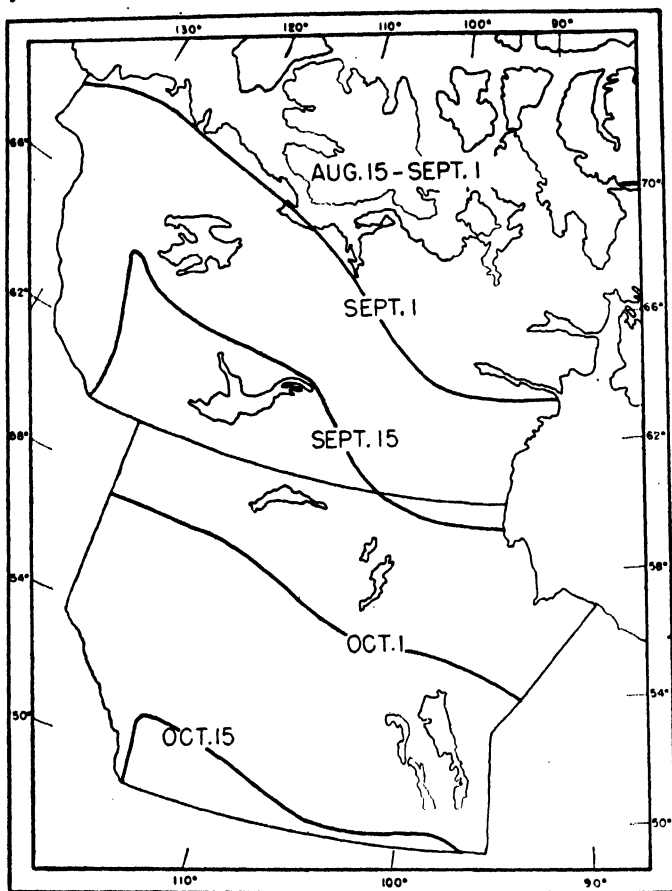


FIG. 2. Isolines for the mean dates of the ending of the vegetative season for the Prairie Provinces and the Northwest Territories.

Only very general statements can be made about the beginning of the vegetative period in the Arctic Archipelago. Mean daily temperatures of  $42^{\circ}$  occur along the southern coasts of Victoria and Baffin Islands during the last week of June, and not at all in the northern fringe of islands. At intermediate positions where the altitude is low and where there is some shelter from the cold winds off the ice-filled channels, they occur (if at all) sometime during the first two weeks of July. Although local conditions often become more favorable to higher temperatures after the middle of July, the steady decrease in the altitude of the sun by this time has an adverse effect; and temperatures never rise above  $42^{\circ}$  for a significant period of time.

Fig. 2 shows isolines for the dates of the ending of the vegetative season. At places in the Archipelago with a growing period warranting consideration, the mean daily temperatures drop below  $42^{\circ}$  sometime during the last two weeks of August. A definite retreat over the mainland is in progress by Sept. 1, the period of growth having come to an end along the Arctic coast from the Mackenzie delta to Coronation Gulf and from the latter place to Chesterfield Inlet by this date. By Sept. 15, it has passed Great Bear Lake, and is approaching the eastern arm of Great Slave Lake and Churchill; by Sept. 30, it has reached the lower valley of Peace River, passed Lake Athabaska and Reindeer Lake, and is approaching Lake Winnipeg; and by Oct. 15 it is practically at the southern borders of the Prairie Provinces. The retreat parallels the lines of latitude more closely than the advance, owing to the higher water vapor content of the air masses from off the Arctic Archipelago and Hudson Bay and the consequent reduction in radiational losses of heat from the surface.

The average length of the growing season at any place may be found from Figs. 1 and 2 by using them to find the dates of the beginning and the ending, respectively, and then counting the number of days between the first and second dates. Values for a number of representative places are given in Table I. Even a casual examination of the two figures shows that the vegetative season varies from 180 to 190 days along the southern border of the Prairie Provinces to only about 90 days along a zone running northwestward from northern Manitoba to the portion of the Arctic coast located to the north of Great Bear Lake. The longest periods in the Arctic Archipelago occur in southern Victoria Island (also in southern Baffin Island) where the coastal

TABLE I

THE AVERAGE LENGTH OF THE VEGETATIVE SEASON, THE AVERAGE NUMBER OF DAY-DEGREES, AND THE AVERAGE DAILY TEMPERATURE FOR THE SEASON IN DEGREES ABOVE  $42^{\circ}$  FOR EACH OF A NUMBER OF REPRESENTATIVE PLACES IN THE PRAIRIE PROVINCES AND THE NORTHWEST TERRITORIES

Station (lat. N.; long. W.)	Vegetative season	Day-degrees	Daily temperature
	days		
Morden ( $49^{\circ} 11'$ ; $98^{\circ} 5'$ )	180	2890	16.0
Lethbridge ( $49^{\circ} 43'$ ; $112^{\circ} 51'$ )	189	2590	13.6
Qu'Appelle ( $50^{\circ} 31'$ ; $103^{\circ} 56'$ )	175	2410	13.7
Scott ( $52^{\circ} 22'$ ; $108^{\circ} 46'$ )	167	2170	13.0
Edmonton ( $53^{\circ} 33'$ ; $113^{\circ} 30'$ )	174	2210	12.7
Norway House ( $53^{\circ} 59'$ ; $97^{\circ} 50'$ )	149	1880	12.6
Beaverlodge ( $55^{\circ} 10'$ ; $119^{\circ} 19'$ )	169	1890	11.2
Ft. Vermilion ( $58^{\circ} 23'$ ; $116^{\circ} 3'$ )	141	1710	12.0
Ft. Chipewyan ( $58^{\circ} 43'$ ; $111^{\circ} 9'$ )	141	1780	12.5
Churchill ( $58^{\circ} 47'$ ; $94^{\circ} 11'$ )	90	740	8.2
Ft. Simpson ( $61^{\circ} 52'$ ; $121^{\circ} 15'$ )	133	1560	11.7
Chesterfield ( $63^{\circ} 20'$ ; $90^{\circ} 43'$ )	63	190	3.0
Coppermine ( $67^{\circ} 45'$ ; $115^{\circ} 5'$ )	70	270	3.8
Aklavik ( $68^{\circ} 14'$ ; $134^{\circ} 50'$ )	95	790	8.3
Cambridge Bay ( $69^{\circ} 5'$ ; $105^{\circ} 0'$ )	63	200	3.2

stations have seasons of about 60 days. Since the coastal waters are cold, somewhat longer periods may exist inland in favorable localities.

It should be noted that many small areas probably exist throughout this region as a whole, where owing to either favorable or unfavorable microclimatic factors, the seasons are either longer or shorter than the ones indicated by the figures. Also, the fact that the values are primarily designed to compare temperature effects on growth should not be overlooked. Across the grasslands in the south, the growing season is often much shorter than indicated because of the lack of moisture, precipitation becoming as important a climatic factor as temperature in limiting growth. Northward, temperature rapidly becomes the predominating factor, and any shortening of the vegetative season from lack of precipitation is insignificant.

The decrease in the length of the vegetative season with increasing latitude does not indicate the corresponding change in the average daily temperature for the season, a quantity that is of some importance in estimating the possibility of maturing certain types of crops (5, pp. 292-307). One way of determining this temperature, referred to the base temperature of  $42^{\circ}$ , is to plot the mean monthly temperatures against the middle dates of the corresponding months, joining the consecutive points by straight lines, drawing the line corresponding to a constant temperature of  $42^{\circ}$ , and measuring the area enclosed by the two graphs. The area is then converted into a quantity that is nothing more than the summation of the differences between the mean daily temperature and  $42^{\circ}$  for each day in the season. This quantity is sometimes referred to as the total number of day-degrees for the season. The daily seasonal temperature in degrees above the base temperature ( $42^{\circ}$ ) is then obtained by dividing this summation by the number of days in the season. Although the value obtained in this way often differs by a small amount from the correct value determined by summing the differences between the mean temperature and  $42^{\circ}$  for each day and dividing this sum by the number of days in the season, it is sufficiently reliable for studies of this type and has the decided advantage that it can be obtained far more quickly. This was done for a number of representative places in the Prairie Provinces and the Northwest Territories. The average length of the vegetative season, the corresponding number of day-degrees, and the average daily temperature of the vegetative season for each place are given in Table I.

This table indicates that the number of day-degrees decreases more rapidly with latitude than the length of the vegetative season. However, at all the places in the Prairie Provinces with a continental type of climate, and even as far north as Ft. Simpson, the average daily temperatures for the growing seasons are substantially constant. At Aklavik the daily temperature is about  $5^{\circ}$  lower than for the Prairies, and is almost the same as for Churchill where the cold waters of Hudson Bay keep the seasonal temperature to a much lower value than the latitude warrants. The combined effects of high latitudes and cold waters are evident by the low seasonal values for Chesterfield and Cambridge Bay.

It is worth noting that the number of day-degrees cannot be used as an exact guide to the possibility of maturing particular crops in a given region, since the heat requirements of a plant vary with a number of other factors, such as the frequency of very high temperatures, the availability of moisture to promote growth, the hours of sunlight, etc. The long hours of sunlight at higher latitudes is often offered as a factor that annuls to some extent the effects of a shorter growing season and a lower seasonal temperature. A partial test of this idea was made from the average dates for the first seeding and the first harvesting of spring wheat at a number of the Dominion Experimental Farms (6, p. 7), and the mean monthly temperatures at these places.

TABLE II

THE AVERAGE LENGTH OF THE MATURITY SEASON FOR SPRING WHEAT AND THE CORRESPONDING NUMBER OF DAY-DEGREES AND THE AVERAGE DAILY TEMPERATURE IN DEGREES ABOVE 42° AT SOME OF THE DOMINION EXPERIMENTAL FARMS IN THE PRAIRIE PROVINCES

Station (lat. N.)	Length of season	Day-degrees	Daily temperature
Morden (49° 11')	100	1650	16.5
Lethbridge (49° 43')	109	1680	15.4
Swift Current (50° 20')	108	1750	16.0
Scott (52° 22')	107	1600	14.9
Beaverlodge (55° 10')	119	1570	13.2
Ft. Vermilion (58° 23')	113	1460	12.9

The appreciable increases in the lengths of the maturity seasons at higher latitudes may result from the decreases in the average seasonal temperatures. However, the known increases in effectiveness of precipitation (the ratio of actual precipitation to evaporation) at these latitudes would have the same effect. The marked decreases in the day-degrees for the maturity seasons suggest the existence of a compensating climatic factor that may well be the increased hours of sunlight.

### The Frost-Free Season

Median dates were used in preference to mean dates for determining the length of the frost-free season. Trials of the two methods showed that the median values give a much better measure of the actual situation at places with a comparatively short temperature record, an extremely early or late frost altering the median date to a lesser extent than the average date. In addition, the median date is exactly the date that is far too frequently interpreted as the average date, namely, the date after or before which one-half of the years will have their last frost for the spring months, or their first frost for the autumn months. For weather stations with long records, the two dates do not differ significantly. No reduction to a normal period was

possible, since very local conditions often give large variations in the dates of frosts at stations quite close to each other.

Isolines, indicating the median dates of the last frost of spring and of the first frost of autumn in terms of days counted from the beginning of the year, are shown in Figs. 3 and 4, respectively. These are drawn for intervals of



FIG. 3. Isolines for the median number of days from the beginning of the year to the last day in spring with frost for the Prairie Provinces and the Northwest Territories.

10 days, a quantity commensurate with the uncertainty of the values and the scale of the maps. Isolines are not shown for the extreme northeastern portion of the mainland and most of the Arctic Archipelago, the available data (both instrumental and visual) indicating that intervals of more than 30 days without frost seldom occur there.

The northward advance of the frost-free season is similar in some respects to that of the vegetative season, the most rapid advance taking place through the drainage basin of the Mackenzie. However, only 40 days is required for the advance to reach the Arctic coast compared with 60 days for that of the

vegetative season. This results in almost a merging of the two seasons at higher latitudes. The physical cause is the long hours of sunlight at the time when the mean daily temperatures have passed  $42^{\circ}$ , and the consequent infrequency of radiational cooling reducing the minimum temperatures to  $32^{\circ}$  or lower. Local anomalies in the rate of advance are mostly due to variations

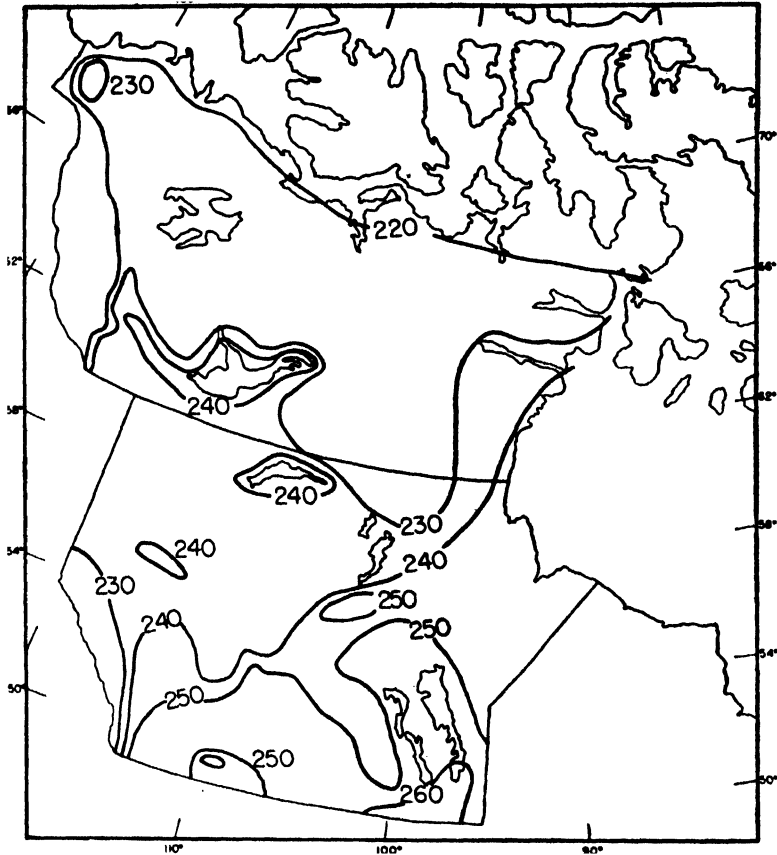


FIG. 4. Isolines for the median number of days from the beginning of the year to the first day in autumn with frost for the Prairie Provinces and the Northwest Territories.

of altitude. A delay of 10 to 20 days, compared with the surrounding country, occurs in the Cypress Hills of southwestern Saskatchewan; and about 10 days in the Riding and Duck Mountains and the Pasquia Hills to the west of the great lakes of Manitoba. In general, the frost-free season enters southern Alberta and Saskatchewan by May 20, crosses the grasslands by May 30, travels to the delta of Mackenzie and to the northern borders of Saskatchewan and most of Manitoba by June 19, and reaches the Arctic coasts by June 29.

The southward retreat of the frost-free season departs appreciably from the pattern followed by the vegetative season, variations of altitude, and the



moderating effects of large bodies of water (now ice free) causing large departures from even an approximate latitudinal retreat. This is most marked on the lowlands to the west of Hudson Bay and in Manitoba, where the lower altitudes and the effects of Hudson Bay and the large lakes of Manitoba cause delays of 10 to 20 days in the incidence of frost. It is also noticeable around Great Slave Lake and Lake Athabaska. Increase of altitude in the Cypress Hills brings the season to an end from 10 to 20 days earlier than in the surrounding country. In a very general way, the season ends along the Arctic coasts by Aug. 8, throughout most of the Northwest Territories by Aug. 18, and the Prairie Provinces by Sept. 10. A small part of southern Manitoba has a frost-free season that does not end until after Sept. 17. It is within this area that the larger fruits are successfully grown.

The length of the frost-free season may be found for any particular locality from Figs. 3 and 4. This is done by finding the days from the beginning of the year to the median date of the spring frost and to the median date of the autumn frost, and subtracting the former quantity from the latter. Across the south of the Prairie Provinces the season varies in length from 90 to 120 days, and in northern Alberta and to the south and west of Great Slave Lake from 70 to 90 days. From Ft. Simpson to the delta of the Mackenzie the season varies in length considerably (depending on local climatic factors) the length at Ft. Norman being 45 days, at Ft. Good Hope 57 days, and at Aklavik 65 days. Along the west coast of Hudson Bay from Churchill to Chesterfield and for some distance inland the season is approximately 70 days. At Coppermine on Coronation Gulf and at Cambridge Bay on the southeast corner of Victoria Island the season is about 60 days. Along all Arctic coasts, encumbered with ice either continuously or at frequent intervals during the summer, a year with 30 or more days free from frost rarely occurs.

Microclimatic effects result in many small areas throughout this region having frost-free seasons both notably longer and shorter than the characteristic lengths for their localities. This has been demonstrated by the observations of Albright and Stoker (2) at Beaverlodge, Alta., where they found that the frost-free season at a hilltop averaged more than three times as long as at a slough, 134 ft. lower, where the cold air drained from the surrounding slopes. At the slough frost occurred in every month of the average year, whereas on the hilltop roses bloomed not infrequently in October. At higher latitudes this drainage of cold air, resulting from radiational cooling at the surface, is not important during June or July because of the short period during the day when the outgoing radiation from the surface exceeds the incoming radiation from the sun and the atmosphere. During August the nights are considerably longer and the drainage of cold air into low-lying flats is more effective in extending the frost-free season on the higher land.

The variabilities of the dates of the last frosts of spring and the first frosts of autumn at a number of representative places were determined by finding the quartile dates for these events. These dates together with the median and extreme dates are listed in Table III. The values for each place are

TABLE III

EXTREME, 25-PERCENTILE, MEDIAN, AND 75-PERCENTILE DATES FOR THE LAST FROSTS OF SPRING AND THE FIRST FROSTS OF AUTUMN AT PLACES WHERE ECONOMIC CROPS ARE EITHER, OR MAY BE, REGULARLY GROWN. THE NUMBER BEFORE EACH STATION REFERS TO A DIAGRAM OF THE SAME NUMBER IN FIG. 5

Station	Spring frosts					Autumn frosts				
	Earliest date	25. percentile date	Median date	75. percentile date	Latest date	Earliest date	25. percentile date	Median date	75. percentile date	Latest date
1 Morden	Ap. 23	My. 15	My. 20	My. 30	Jn. 21	Sp. 8	Sp. 15	Sp. 21	Ot. 1	Ot. 9
2 Brandon	My. 10	My. 27	Jn. 5	Jn. 12	Jl. 1	Ag. 3	Ag. 27	Sp. 6	Sp. 11	Sp. 20
3 Qu'Appelle	Ap. 19	My. 24	Jn. 4	Jn. 9	Jn. 22	Ag. 11	Ag. 27	Sp. 11	Sp. 6	Ot. 1
4 Swift Current	My. 1	My. 23	Jn. 1	Jn. 5	Jn. 22	Ag. 6	Ag. 30	Sp. 13	Sp. 23	Ot. 7
5 Medicine Hat	My. 25	My. 7	My. 14	My. 24	Jn. 6	Ag. 27	Sp. 11	Sp. 16	Sp. 24	Ot. 14
6 Bereas River	My. 16	My. 26	Jn. 6	Jn. 20	Jl. 12	Ag. 14	Ag. 27	Sp. 10	Sp. 18	Ot. 4
7 Dauphin	My. 11	My. 19	Jn. 25	My. 30	Jn. 21	Ag. 25	Sp. 10	Sp. 16	Sp. 26	Ot. 6
8 Saskatoon	Ap. 29	My. 14	My. 22	Jn. 4	Jn. 24	Ag. 17	Sp. 7	Sp. 11	Sp. 20	Ot. 7
9 Edmonton	Ap. 29	My. 22	Jn. 1	Jn. 9	Jl. 2	Ag. 8	Ag. 28	Sp. 7	Sp. 12	Ot. 7
10 Edson	My. 30	Jn. 7	Jn. 15	Jn. 24	Jl. 9	Jl. 16	Ag. 2	Ag. 21	Sp. 3	Sp. 23
11 Beaverlodge	My. 10	My. 22	Jn. 4	Jn. 16	Jn. 27	Ag. 1	Ag. 31	Sp. 8	Sp. 13	Ot. 5
12 Dunvegan	My. 15	My. 29	Jn. 5	Jn. 15	Jn. 28	Jl. 17	Ag. 15	Ag. 27	Sp. 4	Sp. 13
13 Ft. McMurray	Jn. 1	Jn. 6	Jn. 12	Jn. 20	Jn. 29	Ag. 1	Ag. 14	Ag. 24	Sp. 2	Sp. 8
14 Waseca	My. 6	My. 20	Jn. 3	Jn. 7	Jn. 22	Ag. 2	Ag. 24	Sp. 7	Sp. 10	Sp. 17
15 Melfort	My. 10	My. 25	Jn. 5	Jn. 17	Jl. 5	Ag. 10	Ag. 26	Sp. 6	Sp. 13	Sp. 30
16 Norway House	My. 13	My. 26	Jn. 7	Jn. 14	Jl. 4	Ag. 3	Sp. 2	Sp. 10	Sp. 19	Sp. 29
17 Ft. Chipewyan	My. 22	Jn. 3	Jn. 12	Jn. 25	Jl. 1	Jl. 22	Ag. 21	Ag. 31	Sp. 6	Sp. 29
18 Ft. Smith	My. 23	Jn. 5	Jn. 18	Jn. 26	Jl. 9	Jl. 21	Ag. 8	Ag. 23	Sp. 2	Sp. 8
19 Ft. Vermilion	My. 16	Jn. 1	Jn. 11	Jn. 19	Jl. 4	Jl. 20	Ag. 8	Ag. 21	Sp. 3	Sp. 10
20 Hay River	My. 29	Jn. 5	Jn. 9	Jn. 19	Jl. 1	Jl. 31	Ag. 31	Sp. 6	Sp. 17	Sp. 28
21 Ft. Good Hope	My. 24	My. 30	Jn. 10	Jn. 20	Jl. 22	Jl. 25	Ag. 31	Ag. 6	Ag. 16	Ag. 31

Note:—Ap.—April, My.—May; Jn.—June; Jl.—July; Ag.—August; Sp.—September, Ot.—October.

shown graphically in Fig. 5 in such a way as to show also the length of the frost-free season. The upper set of diagrams is for spring frosts, and lower set for autumn frosts. The significant points in each pair of diagrams are as

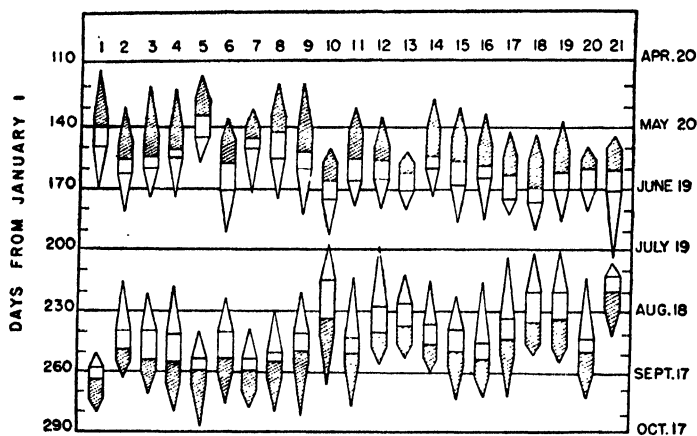


FIG. 5. Diagrams showing extreme, 25-percentile, median, and 75-percentile dates for frosts, and the median lengths of the frost-free seasons at a number of places in the Prairie Provinces and the Mackenzie basin. The numbers at the top of the diagrams refer to place names in Table III.

follows: (a) the vertices, which indicate the earliest and latest observed dates (July frosts ignored at southern stations); (b) the bases of the upper triangles, which indicate the 25-percentile dates or the date before which 25% of the years with observations have had their last spring frost in the case of the upper diagram and their first autumn frost in the case of the lower diagram; (c) the bases of the lower triangles, which indicate 75-percentile dates or the dates before which 75% of the years have had their last spring and their first autumn frost, as the case may be; and (d) the lines separating the shaded from the unshaded areas, which indicate the median or 50-percentile dates. The diagrams for the majority of the places are based on the observations for at least 30 years. For Qu'Appelle, Swift Current, Medicine Hat, and Edmonton, the periods are about 55 years.

From the diagrams it may be seen that the quartile differences, or the differences between the 25- and 75-percentile dates (measured in days) do not show any very characteristic variations with latitude or with locality. The differences vary from two to three weeks. In general, the two dates are not symmetrically distributed about the median date, since the intervals for the second quartile of the spring frosts and the third quartile of the autumn frosts are shorter than the intervals for the third quartile of the spring frosts and the second quartile of the autumn frosts, respectively. This grouping of the frosts is probably due to the rapid changes in temperature that occur at these times of the year,—in the first case from winter to summer values and in the second case from summer to winter values.

The majority of the frosts that take place after the median dates for the late frosts and before the median dates for the early frosts are seldom severe, shelter temperatures rarely dropping more than  $3^{\circ}$  or  $4^{\circ}$  below  $32^{\circ}$ . However, hard frosts that were decidedly unseasonal have occurred occasionally, for example,  $15^{\circ}$  at Edmonton on June 13, 1885;  $21^{\circ}$  at Swift Current on June 2, 1919;  $24^{\circ}$  at Berens River on June 26, 1927;  $18^{\circ}$  at Hay River on June 3, 1930;  $19^{\circ}$  at Ft. Smith on June 1, 1916; and  $23^{\circ}$  at Ft. McMurray on June 9, 1927. Perhaps the most untimely, widespread frost on record occurred on July 24 and 25, 1918, when temperatures as low as  $20^{\circ}$  were recorded at many places in the Peace River country,  $24^{\circ}$  in the park-belt zone of Saskatchewan, and  $30^{\circ}$  across the south of the Prairie Provinces.

The vertical spacing between the shaded sections of each pair of diagrams in Fig. 5 is proportional to the length of the frost-free season. Its steady decrease with increasing latitude is obvious. Edson (10) is an example of a place in the same latitude as Edmonton (9) but some 900 ft. higher. The effects of Great Slave Lake and Lake Athabaska in increasing the frost-free season (especially in the autumn) is apparent by comparing the sets of diagrams for Ft. Chipewyan (17) and Ft. Smith (18); and Hay River (20) and Ft. Vermilion (19), respectively.

It appears from the diagrams in Fig. 5 that exceptionally long frost-free seasons are possible, since the earlier dates of the late frosts may occur in the same year as the later dates for the early frosts. The converse situation could also occur between the late dates for the late frosts and the early dates for the early frosts. Actually, the number of observed instances are infrequent. A few typical examples for a number of places are as follows: (a) Lethbridge with 151 days in 1940 and 70 days in 1902; (b) Medicine Hat with 160 days in 1940 and 92 days in 1917; (c) Ft. McMurray with 100 days in 1940 and 36 days in 1934; (d) Ft. Vermilion with 100 days in 1941 and 47 days in 1931; (e) Aklavik with 107 days in 1938 and 39 days in 1934; and (f) Coppermine with 80 days in 1935 and 31 days in 1939. Extreme differences of this type do not occur where a large body of water acts as a control on the temperature.

Definite evidence that the frost-free season has increased in length during the past 30 years, such as has been found by Kincer (7) for the United States, does not exist. The only weather station with a long weather record that has been affected by the growth of a large city around about it is at Qu'Appelle. Ten-year accumulated sums of the lengths of the frost-free seasons at Qu'Appelle failed to show significant trends in the length.

### Acknowledgment

This work was done as part of a much larger investigation of the climates of the Prairie Provinces and the Northwest Territories. It is financed in part by a grant from the Social Science Research Council of Canada.

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# THE MINERAL REQUIREMENTS FOR PYOCYANIN PRODUCTION<sup>1</sup>

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## Abstract

An investigation of the salt requirements for pyocyanin production by *Pseudomonas aeruginosa* has been carried out, and the mineral balance for maximum pyocyanin production determined. By employing a medium containing the required salt balance, it has been possible to obtain a fourfold increase in pyocyanin production over that obtained on other media usually employed for that purpose.

The ions, Mg, SO<sub>4</sub>, K, PO<sub>4</sub>, and Fe have been found essential to the formation of the pigment. Optimum production of pyocyanin was obtained by the use of 0.04% dipotassium hydrogen phosphate, 2.0% magnesium sulphate septahydrate, and 0.001% ferrous sulphate septahydrate in a medium containing glycerol, glycine, and leucine. In the presence of minimum amounts of Mg and SO<sub>4</sub>, maximum pyocyanin production can be obtained by the addition of increased amounts of either Mg or SO<sub>4</sub>.

As long as no satisfactory synthetic medium capable of providing for the production of pyocyanin by *Pseudomonas aeruginosa* was available, little work could be done on a study of the mineral requirements for the production of this pigment. The establishment of such a synthetic medium (1) has made possible a detailed study of the ionic requirements for pyocyanin formation.

Robinson in 1932 (2) determined the salt requirements for the minimum growth of *P. aeruginosa*, and, in addition, listed some of the common minerals and ions that he found unessential. He stated that both PO<sub>4</sub> and Mg were necessary, while Na, K, Ca, Cl, SO<sub>4</sub>, and CO<sub>3</sub> were unessential. He stated further that the formation of both fluorescein and pyocyanin required only those substances necessary for growth.

In the study of the amino acid requirements for pyocyanin production (1) a salt mixture consisting of magnesium sulphate septahydrate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium chloride, and ferrous sulphate septahydrate was employed in order that the supply of minerals would not be a limiting factor in pigment formation.

In order to determine what mineral constituents of the medium are essential to pyocyanin formation and to establish the concentrations of salts required for optimum yield of the pigment the following experiments were carried out.

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## Method

A synthetic medium that had been shown to be satisfactory for the production of pyocyanin by *P. aeruginosa* was employed as the basal medium. This medium designated as Medium *B* had the following composition:

	%
Glycerol	1.0
Glycine	0.6
<i>l</i> -Leucine	0.6
Magnesium sulphate septahydrate	0.01
Dipotassium hydrogen phosphate	0.01
Potassium dihydrogen phosphate	0.01
Potassium chloride	0.004
Ferrous sulphate septahydrate	0.0004

By omitting each salt respectively from this medium those that are required for pyocyanin formation were determined; by varying the percentage of each salt in the medium, the optimal levels of concentration of the respective salts were ascertained; and finally, by eliminating each respective ion from the medium those that are essential to pigment formation were established.

Throughout this study, water redistilled through glass was employed. All glassware was treated with nitric acid cleaning solution. Reagent quality chemicals were used.

The methods of culturing the organism (*P. aeruginosa* A.T.C. 9027) and extracting the pigment were those previously employed (1). Ten milliliter quantities of media were cultured in 125 ml. Erlenmeyer flasks and the pyocyanin after extraction with chloroform was measured in a Fischer Electrophotometer, employing suitable dilutions of the extracted pigment.

## Experimental

### Essential Salts

The effect of the omission of each of the component salts constituting Medium *B* on growth and pyocyanin formation is given in Table I.

TABLE I  
ESSENTIAL SALTS FOR GROWTH AND PYOCYANIN PRODUCTION

Medium	Pyocyanin*	Growth
Medium <i>B</i>	40.5	++
Medium <i>B</i> minus $K_2HPO_4$	24.0	++
Medium <i>B</i> minus $KH_2PO_4$	26.0	++
Medium <i>B</i> minus $K_2HPO_4$ and $KH_2PO_4$	0	0
Medium <i>B</i> minus $MgSO_4 \cdot 7H_2O$	0	0
Medium <i>B</i> minus KCl	40.0	++++
Medium <i>B</i> minus $FeSO_4 \cdot 7H_2O$	0	++++

\* Pyocyanin expressed as reading on logarithmic scale of Fischer Electrophotometer.

It is clear from Table I that magnesium sulphate and potassium phosphate are essential to growth. The extent of growth obtained and pigment formed is shown to depend on the concentration of potassium phosphate. In the absence of ferrous sulphate maximum growth is obtained with marked production of fluorescin but no elaboration of pyocyanin. It is realized that traces of iron, sufficient for growth, were present as contaminants in the medium. However, the iron present due to contamination was calculated to be less than 0.02 p.p.m. and was shown to be inadequate for pyocyanin production. Potassium chloride would appear to be unessential for either growth or pyocyanin production in this medium.

#### *Optimum Concentrations of Salts*

Although the findings indicated that potassium chloride is not required for pyocyanin production it was thought that different concentrations of the salt might influence the quantitative production of pigment. Employing levels of potassium chloride ranging from 0.0001% to 0.1%, no effect on the production of pyocyanin was detected.

In a detailed study of the potassium phosphate requirements of the organism, dipotassium hydrogen phosphate at varying concentrations was substituted for the two phosphate salts employed in Medium B. The results obtained are recorded in Table II.

TABLE II  
THE EFFECT OF VARYING CONCENTRATIONS OF  
DIPOTASSIUM HYDROGEN PHOSPHATE  
ON PYOCYANIN PRODUCTION

$K_2HPO_4$ , %	Pyocyanin
0.0001	0.0
0.005	12.5
0.01	20.5
0.03	50.0
0.05	43.5
0.07	17.5
0.10	0.0

At a concentration of 0.0001% dipotassium hydrogen phosphate practically no growth was obtained and there was no pyocyanin formation. The maximum amount of pyocyanin occurred at a level of 0.03% dipotassium hydrogen phosphate. At this concentration no fluorescin was produced. At higher concentrations of phosphate the progressive decrease in pyocyanin production was accompanied by a proportional increase in fluorescin. At 0.1% dipotassium hydrogen phosphate there was good growth with marked fluorescin production but no pyocyanin formation.



The influence of magnesium sulphate septahydrate concentration on pyocyanin production is given in Table III.

TABLE III  
THE EFFECT OF VARYING CONCENTRATIONS OF  
MAGNESIUM SULPHATE SEPTAHYDRATE  
ON PYOCYANIN PRODUCTION

MgSO <sub>4</sub> · 7H <sub>2</sub> O, %	Pyocyanin
0.0001	0
0.005	29.5
0.01	45.5
0.05	59.5
0.07	60.1
0.10	61.0
0.50	60.0

Only a slight amount of growth occurred when 0.0001% magnesium sulphate septahydrate was employed. Maximum pyocyanin production was obtained between 0.05% and 0.5% magnesium sulphate septahydrate. No fluorescin was obtained under any of the experimental conditions described in Table III.

A series of concentrations of ferrous sulphate septahydrate were substituted for the 0.0004% of the salt employed in Medium B. The results obtained are given in Table IV.

TABLE IV  
THE EFFECT OF VARYING CONCENTRATIONS OF  
FERROUS SULPHATE SEPTAHYDRATE  
ON PYOCYANIN PRODUCTION

FeSO <sub>4</sub> · 7H <sub>2</sub> O, %	Pyocyanin
0.00001	0
0.00005	9.5
0.0001	19.5
0.0003	33.0
0.0005	45.0
0.005	47.0
0.01	22.0
0.03	0

At a concentration of 0.00001% ferrous sulphate septahydrate, abundant production of fluorescin but no evidence of pyocyanin occurred. However, as the percentage of ferrous sulphate septahydrate approached 0.0005%, fluorescin production decreased proportionally with the increase in pyocyanin. At 0.0005% and at higher concentrations, no fluorescin was observed.

Maximum pyocyanin formation occurred between 0.0005% and 0.005% ferrous sulphate septahydrate. Growth was inhibited in the presence of 0.03% ferrous sulphate septahydrate.

These experiments show that the optimum concentrations of the essential salts for pyocyanin production are 0.03% dipotassium hydrogen phosphate, 0.05% to 0.5% magnesium sulphate septahydrate and 0.0005% ferrous sulphate septahydrate. Since these optimum concentrations were determined in the presence of suboptimal concentrations of the other salts constituting the medium, it was decided to determine the effect of varying the concentrations of each of the individual salts while holding the others at their newly established optima. The salt concentrations employed and the amount of pyocyanin obtained from the respective media are recorded in Table V.

TABLE V  
THE EFFECT OF SALT CONCENTRATION ON PYOCYANIN PRODUCTION

$K_2HPO_4$ , %	$MgSO_4 \cdot 7H_2O$ , %	$FeSO_4 \cdot 7H_2O$ , %	Pyocyanin
0.03	0.1	0.0005	70.5
0.035	0.1	0.0005	69.0
0.04	0.1	0.0005	67.0
0.05	0.1	0.0005	67.0
0.03	0.3	0.0005	77.0
0.03	0.5	0.0005	84.0
0.03	1.0	0.0005	97.0
0.03	2.0	0.0005	100.0
0.03	3.0	0.0005	100.0
0.03	0.1	0.0008	71.0
0.03	0.1	0.001	71.0
0.03	0.1	0.003	77.0
0.03	0.1	0.005	62.0

The results of this experiment show that the concentrations of the salts required for the production of maximum quantities of pyocyanin lie over a wider range than previously demonstrated. The high level of magnesium sulphate septahydrate required for maximum pyocyanin production is to be especially noted. The possibility that contaminating ions present in the magnesium sulphate might be responsible for the results obtained when higher concentrations of this salt were employed was considered. However, magnesium sulphate purified by successive recrystallization from 0.05 *N* nitric acid gave identical results. Possibly owing to the relatively high concentration of iron employed in these media, no fluorescin was formed under any of the conditions outlined in Table V.

On the basis of results recorded in Table V, the optimal concentrations of the salts required for pyocyanin production were now taken as 0.04% dipotassium hydrogen phosphate, 2.0% magnesium sulphate septahydrate, and

0.001% ferrous sulphate septahydrate. Employing the salts at these levels, an experiment similar to that reported in Table V was carried out. The results confirmed the findings established in Table V as to the optimal requirements of the respective salts. A medium consisting of glycerol, glycine, and *L*-leucine in the concentrations used in Medium B, plus dipotassium hydrogen phosphate, magnesium sulphate, and ferrous sulphate, employed at the established optimal concentrations, gave a pyocyanin reading of 88 when a 1 : 3 dilution of the pyocyanin extract was employed, compared with a reading of 40.5 (1 : 1 dilution of pyocyanin extract) employing Medium B. This fourfold increase in pyocyanin production was due entirely to the alteration in the concentrations of the respective salts in the media.

Confirmation of these findings for other strains of the species was obtained when five different cultures were grown in a medium made up of salts at the concentrations established as optimum and in media containing lower and higher amounts of each of the component salts.

It was therefore concluded that, in a medium consisting of glycerol, 1%; glycine, 0.6%; and *L*-leucine, 0.6% the following salt concentrations were essential for optimum production of pyocyanin by *P. aeruginosa*:

Dipotassium hydrogen phosphate	0.04%
Magnesium sulphate septahydrate	2.0 %
Ferrous sulphate septahydrate	0.001%

### *Essential Ions*

While dipotassium hydrogen phosphate, magnesium sulphate septahydrate, and ferrous sulphate septahydrate at certain definite concentrations have been shown to be essential for pyocyanin production the exact nature of the ions essential to pyocyanin formation has not been clearly established.

In order to establish which ions of the various salts in the basic medium required for pyocyanin production are essential, media in which one of each of the respective ions was omitted were prepared. Control media in which dipotassium hydrogen phosphate was replaced by potassium chloride and calcium monohydrogen phosphate and in which magnesium sulphate and ferrous sulphate were replaced by magnesium chloride, calcium sulphate, and ferric chloride were also prepared. The basal medium employed in this experiment was made up of glycerol, 1.0%; glycine, 0.6%; *L*-leucine, 0.6%; dipotassium hydrogen phosphate, 0.03%; magnesium sulphate septahydrate, 0.05%; and ferrous sulphate septahydrate, 0.0005%.

The pyocyanin readings of extracts obtained from these media are recorded in Table VI.

The findings show that each of the five ions employed is essential to pyocyanin formation. Good growth occurs in the absence of K, SO<sub>4</sub>, or added Fe. In the absence of added Fe, fluorescein production without pyocyanin formation is abundant confirming the findings recorded in Table I.

As mentioned previously sufficient iron is present as contamination to support the growth of *P. aeruginosa* for Waring and Werkman (3) showed

TABLE VI  
IONS ESSENTIAL TO PYOCYANIN FORMATION

Salt content of medium	Growth	Pyocyanin
1. $K_2HPO_4$ , $MgSO_4$ , and $FeSO_4$	++++	67.0
2. $K_2HPO_4$ replaced by $KCl$ and $CaHPO_4$	++++	43.5
3. $MgSO_4$ and $FeSO_4$ replaced by $MgCl_2$ , $CaSO_4$ , and $FeCl_3$	++++	56.5
4. $PO_4$ omitted, $K_2HPO_4$ replaced by $KCl$	+	0
5. $K$ omitted, $K_2HPO_4$ replaced by $CaHPO_4$	++++	0
6. $SO_4$ omitted, $MgSO_4$ and $FeSO_4$ replaced by $MgCl_2$ and $FeCl_3$	++++	0
7. $Mg$ omitted, $MgSO_4$ replaced by $CaSO_4$	++	0
8. $Fe$ omitted, $FeSO_4$ omitted	++++	0

that although  $Fe$  was necessary for the growth of this organism maximum growth would occur at 0.08 p.p.m. and half maximum growth at 0.02 p.p.m. It was calculated that without added iron the basal medium contained slightly less than 0.02 p.p.m. of iron and therefore would be expected to support the growth of *P. aeruginosa*.

The possibility of replacing certain of the essential ions was then considered. Unsuccessful attempts were made to substitute sodium and ammonium for potassium; manganese, zinc, or cobalt for magnesium; arsenate for phosphate; and copper for iron. In a complete medium cobalt inhibits growth and ammonium, manganese, zinc, arsenate, and copper inhibit to varying degrees the production of pyocyanin.

The marked increase in pyocyanin formation obtained with the increased concentrations of magnesium sulphate septahydrate warranted further investigation in order to ascertain whether or not increased pyocyanin production was due merely to increased growth, and also to demonstrate if possible the respective roles played by  $Mg$  and  $SO_4$  ions in stimulating pyocyanin production.

Cultures grown in media containing a range of concentrations of magnesium sulphate septahydrate were tested for the amount of pyocyanin produced as well as for total bacterial nitrogen. The results indicated that the growth curve levelled off at 0.5% magnesium sulphate septahydrate while the pyocyanin curve continued to increase up to a 3.0% concentration of magnesium sulphate septahydrate.

By adding  $Mg$  and  $SO_4$  ions separately in the form of magnesium chloride and sodium sulphate a more detailed picture of the relative effect of the respective ions was obtained. In the presence of 0.06% sodium sulphate, increasing amounts of  $Mg$  from 0.1% to 3.0% magnesium chloride gave tremendous increases in pyocyanin as well as increased growth. In the

presence of 0.1% magnesium chloride increases in  $\text{SO}_4$  from 0.06% to 1.8% sodium sulphate gave similar increases in pyocyanin production but no increase in growth. These findings are in agreement with the previous observations that the  $\text{SO}_4$  ion is required for pyocyanin production and is not essential for growth whereas Mg is essential for the optimum growth of *P. aeruginosa*. Thus Mg appeared to increase pyocyanin production by stimulating growth whereas  $\text{SO}_4$  improves pigment production without affecting growth.

Although both Mg and  $\text{SO}_4$  are essential to pyocyanin production, it would appear that one ion may in a measure substitute for the other in a medium designed for maximum pyocyanin production. Equal amounts of pyocyanin are produced in media containing either 0.1% magnesium chloride and 1.8% sodium sulphate; 3.0% magnesium chloride and 0.06% sodium sulphate; or 3.0% magnesium sulphate septahydrate.

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## DETERMINING SEED SIZE BY A VOLUMETRIC MEASURE<sup>1</sup>

BY BEATRICE E. MURRAY<sup>2</sup>

### Abstract

A method for determining seed size of flax by a volumetric measurement was devised as an alternative to weighing the seed. The volumetric or 'displacement' measurement can be expressed by means of a factor, in terms of 1000 kernel weight in grams. The 'displacement' method is more rapid than weighing the seed.

### Introduction

In the breeding of flax, seed size is a factor of importance. Size of seed is usually determined on the basis of the 1000 kernel weight in grams. If many lines are to be tested, considerable time is consumed in making accurate weight determinations, using either the analytic or torsion balance, as the variations in weight are small. The volumetric or 'displacement' method was devised for the purpose of increasing efficiency in this operation.

### Materials and Methods

The method of determining seed size consisted of measuring the volume of liquid displaced by a sample of 100 seeds in a glass tube 7 mm. in diameter and 16 cm. long. The tube was clamped in a vertical position to an 18 in. iron stand, using a Bunsen clamp with one flat and one V-shaped jaw. This tube, open at both ends, was used to hold a column of liquid. A stopper placed in the bottom held the liquid while the measurements were taken and enabled quick emptying and clearing of the tube. A millimeter scale was fastened with the tube in the Bunsen clamp in such a way as to allow the scale to be read through the tube.

Ethyl alcohol was used as the displacement liquid as it has a low surface tension that allowed the flax seeds to drop freely to the bottom of the tube. The alcohol quickly evaporated from the seeds and tests showed that it had no effect on germination. The alcohol could be reused when the flax seeds were strained out.

The procedure in making the seed size determinations was to place about 1 cc. of ethyl alcohol in the tube by means of a pipette. One hundred seeds were then counted out as this allowed time for the liquid to drain down from the sides of the tube. To ensure a minimum of error in measurement a plunger was used to remove any liquid adhering to the sides of the tube. The height of the column of liquid was then measured in millimeters. Following this the seeds were dropped through a small funnel into the tube and the

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height of the liquid column was again measured. The difference between the two measurements was the 'displacement' value for the seed size of the sample. When the stopper was removed the contents drained freely from the tube.

It was found that two 'displacements' could be obtained from the use of 1 cc. of alcohol. This was done by using the final or 'displacement' reading of the first sample as the base reading of the second sample. Considerably faster recording of 'displacement' was obtained by this procedure.

TABLE I

DATA ON DISPLACEMENT VALUES, CALCULATED KERNEL WEIGHTS, AND  
THE 1000 KERNEL WEIGHT OF 40 FLAX LINES

Flax line number	Displacement values				Factor	Calculated kernel wt.				1000 kernel weight, gm.
	Sample numbers			Mean value		Sample numbers			Mean value	
	1	2	3			1	2	3		
1	23.6	23.7	23.9	23.7	.224	5.1	5.1	5.1	5.1	5.3
2	31.2	30.1	30.9	30.7	.212	6.7	6.4	6.6	6.6	6.5
3	24.7	26.0	25.5	25.4	.209	5.3	5.6	5.5	5.5	5.3
4	28.0	28.8	28.8	28.5	.211	6.0	6.2	6.2	6.1	6.0
5	28.7	28.5	28.1	28.4	.211	6.1	6.1	6.0	6.1	6.0
6	27.5	27.0	27.3	27.3	.220	5.9	5.8	5.8	5.8	6.0
7	25.6	25.8	25.2	25.5	.208	5.5	5.5	5.4	5.5	5.3
8	24.7	25.0	25.8	25.2	.218	5.3	5.4	5.5	5.4	5.5
9	28.8	29.2	28.3	28.8	.208	6.2	6.2	6.1	6.2	6.0
10	30.6	30.8	31.2	30.9	.217	6.5	6.6	6.7	6.6	6.7
11	30.3	29.9	29.5	29.9	.207	6.5	6.4	6.3	6.4	6.2
12	27.3	27.8	26.7	27.3	.212	5.8	5.9	5.7	5.8	5.8
13	29.0	28.6	28.3	28.6	.203	6.2	6.1	6.1	6.1	5.8
14	24.4	24.7	24.9	24.7	.231	5.2	5.3	5.3	5.3	5.7
15	24.0	22.8	22.5	23.1	.225	5.1	4.9	4.8	4.9	5.2
16	25.9	26.1	25.7	25.9	.220	5.5	5.6	5.5	5.5	5.7
17	26.0	26.4	25.4	25.9	.224	5.6	5.6	5.4	5.5	5.8
18	22.0	22.8	23.0	22.6	.208	4.7	4.9	4.9	4.8	4.7
19	24.5	24.4	23.3	24.1	.207	5.2	5.2	5.0	5.1	5.0
20	28.9	28.5	28.9	28.8	.215	6.2	6.1	6.2	6.2	6.2
21	27.0	27.0	26.6	26.9	.212	5.8	5.8	5.7	5.8	5.7
22	31.0	31.0	30.7	30.9	.210	6.6	6.6	6.6	6.6	6.5
23	29.1	29.2	29.1	29.1	.213	6.2	6.2	6.2	6.2	6.2
24	28.7	28.8	29.0	28.8	.219	6.1	6.2	6.2	6.2	6.3
25	27.6	28.2	27.3	27.7	.217	5.9	6.0	5.8	5.9	6.0
26	31.1	30.3	31.0	30.8	.211	6.7	6.5	6.6	6.6	6.5
27	31.6	32.3	32.6	32.2	.211	6.8	6.9	7.0	6.9	6.8
28	25.0	24.9	25.5	25.1	.227	5.4	5.3	5.5	5.4	5.7
29	25.8	27.0	26.3	26.4	.208	5.5	5.8	5.6	5.6	5.5
30	29.2	28.4	27.7	28.4	.211	6.2	6.1	5.9	6.1	6.0
31	29.6	31.3	30.3	30.4	.220	6.3	6.7	6.5	6.5	6.7
32	26.7	27.7	25.3	26.6	.218	5.7	5.9	5.5	5.7	5.8
33	28.2	30.2	29.9	29.4	.204	6.0	6.5	6.4	6.3	6.0
34	26.6	27.6	27.5	27.2	.213	5.7	5.9	5.9	5.8	5.8
35	28.6	28.6	28.4	28.5	.218	6.1	6.1	6.1	6.1	6.2
36	23.5	23.2	23.6	23.4	.214	5.0	5.0	5.1	5.0	5.0
37	25.2	26.3	26.1	25.9	.220	5.4	5.6	5.6	5.5	5.7
38	29.0	29.5	28.4	29.0	.214	6.2	6.3	6.1	6.2	6.2
39	27.7	29.0	28.0	28.2	.213	5.9	6.2	6.0	6.0	6.0
40	23.0	23.3	23.2	23.2	.216	4.9	5.0	5.0	5.0	5.0

As seed size is generally expressed in terms of 1000 kernel weight in grams it was desirable to determine a relationship between these two methods of obtaining size of seed. The data on 40 flax lines were used to compare the methods. 'Displacements' were taken on three 100 kernel samples of each of the 40 lines. The mean displacement value of the three samples was calculated as this value tended to offset the extremes of measurement. The 1000 kernel weight was computed from the total weights of three lots of 200 seeds each, which is the accepted procedure in the Field Husbandry Department. The results of the comparison of the two methods are given in Table I.

To get the relationship of 'displacement' and 1000 kernel weight as a measure of seed size, a factor was obtained for each line as shown in Table I. This factor was obtained by dividing the 1000 kernel weight of each line by its mean 'displacement' value. It was necessary to find a factor common to both methods of seed size determination. The average of the factors of the 40 lines gave a mean factor of 0.214. The 'calculated kernel weight' was then obtained for each line by multiplying the 'displacement' value by the factor 0.214. In order to measure the variation between the two methods, the calculated kernel weight was determined for each line for all three 'displacement' values as well as for the mean.

The correlation between 1000 kernel weight and the calculated kernel weight of the three different samples and the mean was determined. The correlation coefficient for each determination is given in Table II.

TABLE II

THE CORRELATION BETWEEN 1000 KERNEL WEIGHT IN GRAMS AND THE CALCULATED KERNEL WEIGHT OF EACH OF SAMPLES 1, 2, AND 3 AND THE MEAN

Factors correlated: 1000 kernel weight and calculated kernel weight	Correlation coefficient	$t = \frac{r \times \sqrt{n-2}}{\sqrt{1-r^2}}$	5% point
Sample 1	.94	17.09	2.02
Sample 2	.93	15.67	2.02
Sample 3	.94	17.09	2.02
Mean	.95	18.88	2.02

There was a very high correlation between the two methods, the least variation being shown, as would be expected, between the mean 'calculated kernel weight' and the actual 1000 kernel weight in grams. The average difference between the calculated and the actual 1000 kernel weights was 0.135 gm. or 2.31% of the mean 1000 kernel weight of 5.85 gm. This difference is very small compared with the least significant differences usually accepted in comparing varieties and lines of flax.

The high degree of association between 1000 kernel weight and 'displacement' shows that 'displacement' could be used as an alternative method for



determining seed size of flax samples. The 'displacement' method could conveniently be used when scales for taking the weight of seed are not available. It was found to be more rapid than the analytical balance as considerable time was lost in determining weight by the latter method because variations in weight are small in flax. In using the torsion balance slight variations are less apparent owing to the small weight of each seed and the frictional drag of the scale.

In the 'displacement' method small variations can be measured, as the 'displacement' scale gives a greater number of divisions than is generally used when obtaining 1000 kernel weight with standard equipment. With the new method it may therefore be legitimate to use smaller or fewer samples for computing seed weight.

### **Conclusions**

The 'displacement' method can be used for determining size of seed of flax samples. The seed size of a large number of samples can be determined accurately in less time by the new method than when using scales for weight determination. The displacement method is more efficient than taking kernel weight as smaller or fewer samples were found to give a good measure of size. The greater numerical differences in displacement values as compared with 1000 kernel weights gave a greater range for measurement. The new method may be useful in determining seed size of other small seeded crops.

### **Acknowledgments**

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# ACTINOMYCIN FROM A NEW *STREPTOMYCES*<sup>1</sup>

BY PAUL C. TRUSSELL<sup>2</sup> AND EDWIN M. RICHARDSON<sup>3</sup>

## Abstract

A heretofore undescribed isolate of *Streptomyces*, capable of producing actinomycin in submerged culture, has been studied. The antibiotic substance has been identified chemically and biologically as actinomycin.

## Introduction

Waksman and Tishler (4) stated that, among more than 250 strains of actinomycetes isolated and tested by them, only *S. antibioticus* was able to produce typical actinomycin. Further studies by Waksman *et al.* (3) on 10,000 isolated cultures of actinomycetes revealed only two species or strains of the genus *Streptomyces* other than *S. antibioticus* capable of producing actinomycin.

During the examination of microorganisms from natural sources for antimicrobial activity a *Streptomyces* has been isolated that readily produces actinomycin in submerged culture on an inexpensive medium. This *Streptomyces* isolate, tentatively designated A9-6, has been found to exhibit cultural characters distinct from *Streptomyces antibioticus* (5) (originally named *Actinomyces antibioticus*), *Streptomyces* S-4, and *Streptomyces* 36-G, the only species or strains previously reported as producing actinomycin.

## Experimental

### *Comparison of A9-6 and the Known Actinomycin-producing Streptomyces*

A comparison was made of the growth characters of A9-6 and *S. antibioticus* on various substrates. Results are given in Table I. On all the diagnostic media, A9-6 was distinct in its manner of growth from that recorded for *S. antibioticus*. In contrast to *S. antibioticus*, A9-6 failed to liquefy gelatin, produced aerial mycelia on potato and carrot plugs, caused clearing of litmus milk, and did not form a dark pigment when grown on peptone agar.

Morphological distinctions were seen in the conidiophores, which in *S. antibioticus* are arranged in clusters with no spirals, whereas those of A9-6 are commonly spiralled and exhibit whirl formation.

The sporulating aerial mycelium of A9-6 also differentiates it from *Streptomyces* 36-G, which produces straight conidiophores arranged irregularly on the aerial mycelium (3). A further distinguishing feature is that 36-G is non-chromogenic, while A9-6 is chromogenic.

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TABLE I

COMPARISON OF CULTURAL CHARACTERS OF A9-6 AND *S. antibioticus*

Medium	<i>S. antibioticus</i> *	A9-6
Gelatin	Dark brown growth on surface; patches of gray aerial mycelium. Dark pigment, diffusing into unliquefied gelatin. Liquefaction slow at first, then rapid	Slow growth around sides of tube, some subsurface, grayish white. No aerial mycelium. No pigment. No liquefaction
Potato plug	Folded, brown growth, with thin black ring on plug, fading into bluish tinge. No aerial mycelium	Fairly abundant, raised, thick, wrinkled growth in 48 hr. becomes gray-brown with beads of moisture. Gray-black spores. Orange pigment. Aerial mycelium present
Carrot plug	Cream-colored to faint brownish growth. No aerial mycelium. No pigment	Fairly abundant, slightly raised growth in 48 hr. becomes gray-brown with black spores. Drops of moisture. Orange pigment. Aerial hyphae as short spirals
Litmus milk	Thick brownish ring on surface of milk. Mouse gray aerial mycelium with greenish tinge; growth becomes brown. No reaction change; no coagulation; no clearing; whitish sediment at base of tube. Old cultures: heavy growth ring at surface, heavy precipitation; liquid brownish to black in upper portion	Gray-white to gray-green flakes adhering to sides of tube at surface. No aerial mycelium Reaction slightly alkaline; almost complete clearing in five days; grayish white sediment. In old cultures: liquid red-purple to red-brown
Czapeks' agar	Thin, whitish growth. Thin gray aerial mycelium	Scant growth, close to surface, grayish white. Short gray-brown aerial mycelium. Sporophores in whirls, some in short spirals. Spores elliptical
Peptone agar	Production of dark pigment at early stage of growth very characteristic. Growth brownish, thin, with yellow-gray to yellow-green aerial mycelium	Abundant, raised, white growth in 48 hr. Yellow to orange on under surface

\* As described by Waksman and Woodruff (5).

A9-6 is unlike *Streptomyces* 4-S, which is allied to *S. antibioticus* in some of its morphological and cultural characters (1), in that A9-6 fails to produce a deep black zone in the aerial mycelium on synthetic media.

#### Preparation of Antibiotic Substance

The newly isolated *Streptomyces* A9-6 was grown by standard submerged culture methods on a number of media composed of glucose, salts, and complex organic nitrogen. The medium employed most extensively contained: glucose, 1%; soymeal, 1%; sodium chloride, 0.5%; and sodium nitrate, 0.1%.

The organism was grown in aerated fermenters of 10 liter capacity. Maximum yield of the antibiotic was obtained following four to five days' incubation,

at which time the ferment was yellow-orange in color. The thallus was then removed by filtration and the filtrate subjected to purification according to the procedure of Tishler (2). A diethyl ether soluble, petroleum ether insoluble, material was obtained as red crystals following chromatographic treatment and precipitation from hot acetone by ether and subsequently from hot ethyl acetate.

Tests were made on the crystalline material to determine its identity with actinomycin.

### Identity Tests

Like actinomycin (5), the antibiotic material in broth filtrates of A9-6 is removable by activated carbon; it resists 100° C. for 30 min., and it is extractable by diethyl ether and chloroform. Like actinomycin, the biologically active material of A9-6 is stable to acid (pH 1) but unstable to 0.1 *N* sodium hydroxide.

Specific rotation values ( $(\alpha)_D^{25}$ ) of  $-260^\circ$  and  $-286^\circ$  were obtained for two impure crystalline preparations from A9-6. These compare with a value of  $-320^\circ$  for pure actinomycin (4). The melting point of one sample was 248° to 250° C. with decomposition, compared with decomposition at 250° C. (Waksman and Tishler (4)). On reductive acetylation the substance behaved in a similar manner to actinomycin.

Visible and ultraviolet light absorption ( $E_{1\%}^{1\text{cm.}}$ ) for one preparation from A9-6 showed two maxima, one of 170 at 446  $m\mu$  and another of 265 at 236  $m\mu$ . Another preparation gave maxima of 160 at 446  $m\mu$  and another of 230 at 242  $m\mu$ . These compare with maxima of 200 at 450  $m\mu$  and of 216 at 241  $m\mu$  for actinomycin (4).

TABLE II

SENSITIVITY OF BACTERIA TO PURE ACTINOMYCIN AND TO A PREPARATION FROM A9-6 CULTURE FILTRATE

Concentration per 10 ml. agar	<i>Bacillus mycoides</i>		<i>Bacillus subtilis</i>		<i>Sarcina lutea</i>		<i>Escherichia coli</i>	
	A	B	A	B	A	B	A	B
5 $\mu\text{gm.}$	0	0	0	0	0	0		
1 $\mu\text{gm.}$	0	0	0	0	0	0		
0.5 $\mu\text{gm.}$	0	0	0	0	0	0		
0.25 $\mu\text{gm.}$	1	1	1	1	0	0		
0.10 $\mu\text{gm.}$	3	3	3	3	0	2		
0.05 $\mu\text{gm.}$	3	3	3	3	3	3		
2 mgm.							0	0
1 mgm.							3	3

NOTE: Growth on incubation of plates for 48 hr., at 28° or 37° C.; 0 = none, 1 = trace, 2 = fair, 3 = good.

A = pure actinomycin (Data of Waksman and Tishler (4)).

B = preparation from A9-6.

Bacterial sensitivity was determined by the agar plate-streak method on purified material from the culture filtrate of A9-6, with Waksman's strains of *Bacillus mycoides*, *Bacillus subtilis*, *Escherichia coli*, and *Sarcina lutea*. As may be seen in Table II, the bacterial sensitivity for the antibiotic from A9-6 corresponded to that found for actinomycin (4). The three Gram-positive species were highly susceptible to both antibiotics, whereas *Escherichia coli* was relatively resistant.

TABLE III

ACUTE TOXICITY TO MICE OF A9-6 PURIFIED MATERIAL (INTRAVENOUS INJECTION OF SIX MICE AT EACH DOSAGE)

Dose, μgm./gm.	Number dead after:				
	1 day	2 days	3 days	9 days	14 days
0.15	0	0	0	0	0
0.25	0	0	0	2	2
0.50	1	3	6		
1.00	4	6			
2.00	6				

The acute toxicity in mice of an A9-6 preparation was found to parallel that of actinomycin, which is lethal at a dosage of 10 μgm. subcutaneously or intraperitoneally (4). Intravenous injection of 10 μgm. of purified material from A9-6 culture filtrate was lethal to 20 gm. mice (Table III).

### Discussion

*Streptomyces* A9-6, which has been found to differ from actinomycin-producing organisms previously described, elaborates a substance that is very similar to the antibiotic produced by *S. antibioticus*. The crude culture filtrate of A9-6 may be processed in the same manner as that of *S. antibioticus*. Although the spectrophotometric data and rotation of the material from A9-6 are not identical with those of pure actinomycin, they approximate them and differences may be attributed to impurity of the preparations from A9-6. With respect to animal toxicity and bacterial sensitivity, actinomycin and the antibiotic substance from A9-6 are identical. It therefore appears that A9-6 is a previously undescribed strain of *Streptomyces* capable of producing actinomycin in submerged culture.

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# THE ISOLATION OF A BLUE FLUORESCENT COMPOUND SCOPOLETIN, FROM GREEN MOUNTAIN POTATO TUBERS, INFECTED WITH LEAF ROLL VIRUS<sup>1</sup>

BY W. A. ANDREAE<sup>2</sup>

## Abstract

A method has been described for the isolation of a crystalline, fluorescent compound that occurs in Green Mountain potato tubers during the first year of infection with leaf roll virus. The substance was identified as 7-hydroxy-6-methoxy 1 : 2 benzopyrone (scopoletin), previously isolated by Best from tobacco plants, infected with tomato spotted wilt virus.

## Introduction

Several workers (1, 5, 6), have shown that in certain varieties the cut surface of tubers, infected with leaf roll virus, exhibit a bright blue fluorescence. Sanford and Grimble (6) have associated the occurrence of this fluorescence with the presence of phloem necrosis in the infected tubers. While the leaf roll disease is transmitted from the tuber to the developing plant, the symptoms of phloem necrosis and fluorescence in the tuber are not apparent after the first year of infection. We became interested in the possible role of the fluorescent principle in virus infection and attempted its isolation and identification. A similar fluorescent phenomenon had previously been observed by Best (2) in tobacco plants. This worker found that there was a slight fluorescence in healthy tobacco plants that became very pronounced on infection with tomato spotted wilt virus. The same author published later (3) a procedure by which this fluorescent substance could be isolated in crystalline form and identified it as 7-hydroxy-6-methoxy 1 : 2 benzopyrone, scopoletin. We have modified the procedure of Best, applied it to potato tissues and obtained the same compound from potato tubers, infected with leaf roll virus.

## Experimental

Green Mountain potato tubers that carried the symptoms of phloem necrosis were bisected in the dark and examined in ultraviolet light from a 100 watt BH4 mercury arc. The bright fluorescent portions of about 50 tubers were cut off. As soon as 400 gm. of the cut tissue was obtained, 250 ml. of a dilute solution of sulphuric acid (about 0.05 *N* with a pH of 3 to 4) were added and the whole was finally ground in a Waring blender. This procedure was repeated until a total of about three liters of pulp was obtained. The mash was drained and squeezed through cheesecloth and the liquid centrifuged to free it from all suspended matter. The pulp was discarded and 400 ml. portions of the aqueous extract were shaken up four times in succession with

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Contribution from the Department of Biology, University of New Brunswick, Fredericton, N.B. and the Provincial Department of Agriculture, New Brunswick.

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40 ml. of chloroform. The substance was next extracted from the combined chloroform washings. Portions of the chloroform extract (300 ml.) were shaken up five times in succession with 50 ml. of ammonium hydroxide ( $M/100$  with a pH of 10). The combined aqueous extracts were acidified by adding just enough normal sulphuric acid to bring the pH value to 3 to 4. The end point could be very easily recognized by the sudden disappearance of the intense blue fluorescence. Finally the fluorescent substance was extracted from 300 ml. portions of the acidified aqueous extract by five successive washings with 50 ml. of benzene. The benzene extracts were bulked, dried over neutral calcium chloride, filtered, and chromatographed on Alumina 80-200 MM (Alumina Adsorption, Fisher Scientific Co.). The adsorption column was first washed with benzene and the substance then eluted with peroxide-free ethyl ether. As soon as the fluorescent substance appeared in the eluate, the fraction was collected separately and taken down to dryness under reduced pressure at room temperature. The crystals (about 20 mgm.) were dried in a desiccator and stored in the dark. All operations from the time the tubers were bisected to the final crystallization were carried out in the dark or in subdued light, as the substance is sensitive to light.

The substance crystallized from ether as colorless needles and fluoresced when irradiated in the ultraviolet light. The melting point was determined by two different methods. A value of  $206^{\circ}\text{C}$ . was obtained when the temperature was measured with a thermocouple on the heated stage of a microscope. Subsequent determinations were carried out with a thermometer in a heating bath, employing a capillary melting point tube. The results are reported with their stem corrections. By this method, a second sample showed a melting point at  $203^{\circ}\text{C}$ . A sample of scopoletin, isolated from the roots of *Gelsemium sempervirens*, was kindly supplied by Dr. Léo Marion, National Research Council, Canada, and showed a melting point at  $204.5^{\circ}$  to  $205^{\circ}\text{C}$ . A mixed sample from these two sources gave a distinct melting point at  $203^{\circ}$  to  $204^{\circ}\text{C}$ . Since there was no depression of the melting point these substances are probably the same. Best (3) obtained a melting point of  $206^{\circ}\text{C}$ . with the material that he isolated from tobacco plants.

An aqueous solution of the pure substance at pH 7 fluoresced moderately in daylight while an alkaline aqueous solution showed a strong fluorescence. Boiling did not destroy the fluorescence and in acid solution the fluorescence was scarcely perceptible. An aqueous solution of the substance turned green when ferric chloride was added. On addition of potassium permanganate the substance gave rise to a blue color that turned yellow on alkalization. The crystals dissolved in concentrated nitric acid producing a yellow red solution; on addition of ammonia the color became blood red.

Best (3) has published the molecular extinction curve of the compound in ethyl alcohol that is reproduced in Fig. 1. We too have measured the molecular extinction curve in the same medium using a Beckman spectrophotometer. The readings are included in Fig. 1. Maxima occurred at  $2290\text{ \AA}$ , log E 4.20 ( $2285\text{ \AA}$ , log E 4.25 Best's values);  $2540\text{ \AA}$ , log E 3.72;

2980 Å, log E 3.77; 3460 Å, log E 4.12 (3435 Å, log E 4.14, Best's values). The two molecular extinction curves agree well although our curve shows two small additional maxima that were not reported by Best.

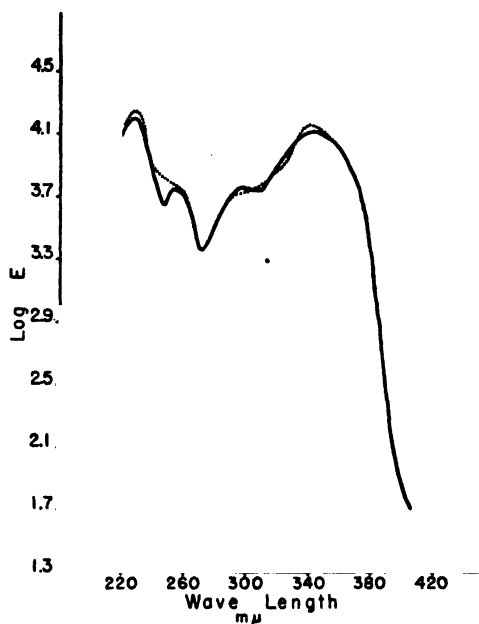


FIG. 1. Molecular extinction curve in ethyl alcohol of the blue fluorescing compound isolated from leaf roll infected potatoes (solid line). The dotted line refers to Best's sample from tobacco infected with tomato spotted wilt and is given only where the two curves do not coincide.

From the similarity of the physical and chemical properties of this compound with those described by Best we have concluded that the substance isolated from Green Mountain potato tubers infected with leaf roll virus is identical with the substance isolated from tobacco plants infected with tomato spotted wilt and is 7-hydroxy-6-methoxy 1 : 2 benzopyrone, scopoletin. When the procedure was applied to healthy Green Mountain tubers or to tubers past the first year of leaf roll infection, only very small amounts of the crystalline material could be obtained.

Lewis and Doty (4) partially characterized the physical and chemical properties of a blue fluorescent compound that they found to be involved in the graying or blackening process of white potatoes when the potatoes were boiled. This colorless, fluorescing pigment precursor was precipitated by ether, could be converted to a black pigment at temperatures above 35° C. or by rendering the solution alkaline, and had an absorption spectrum that showed only one characteristic maximum at 2800 Å. Evidence for the presence of a carbohydrate and a nitrogenous grouping were reported by the authors. This fluorescent substance is therefore chemically different from the one isolated by us.



Investigations on the physiological role, the origin, and the fate of scopoletin in the potato plant are in progress.

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# AUTOGENOUS NECROSIS IN TOMATOES IMMUNE FROM *CLADOSPORIUM FULVUM* COOKE<sup>1</sup>

BY ARTHUR N. LANGFORD<sup>2</sup>

## Abstract

The complication of a tomato breeding project by the occurrence, in lines carrying immunity from *Cladosporium fulvum* Cooke physiologic races 1 to 4, of a spontaneous and destructive necrosis, chiefly of the foliage, has been described.

The autogenous nature of this necrosis has been established thoroughly and the simple Mendelian nature of its inheritance determined. Immune plants, possessing the factor  $Cf_p$ , are potentially necrotic if they are homozygous with respect to the factor  $ne$ , found in *Lycopersicon esculentum* Mill., but remain non-necrotic if homozygous or heterozygous with respect to its dominant allele,  $Ne$ , found in *L. pimpinellifolium* (Jusl.) Mill. Plants susceptible to *C. fulvum* are invariably non-necrotic.

The visible expression of the gene combination  $Cf_p, ne$  has been shown to vary widely according to the environmental conditions, seasonal variations in the severity of necrosis being particularly striking.

The factor  $ne$  has been located on Chromosome I by three-point experiments.

Necrosis appeared in derivatives of crosses between *L. pimpinellifolium* and all the varieties of *L. esculentum* tested.

The data indicate the presence, in *L. pimpinellifolium*, of modifying factors that reduce the severity of necrosis occurring in the presence of the gene combination  $Cf_p, ne$ . Evidence has also been presented that one or more factors on Chromosome VII, closely associated with the factor  $H$  (smooth stem), which occurs in *L. pimpinellifolium*, may prevent the development of necrosis in immune plants of genotype  $ne/ne$ , but the analysis of this phase of the problem is incomplete.

Necrosis is considered to be the visible expression of an incompatibility between  $Cf_p$  and a chromosome complex derived very largely from *L. esculentum*. Speculations concerning the possible evolution, in *L. pimpinellifolium*, of immunity from *C. fulvum* physiologic races 1 to 4 and of freedom from necrosis are outlined.

The bearing of the results of these studies upon projects for the breeding of tomatoes resistant to *C. fulvum* is discussed, particularly in the light of the discovery of races of this fungus that are capable of infecting such varieties as Vetomold, which is immune from races 1 to 4.

## Introduction

The author's original investigations of the parasitism of *Cladosporium fulvum* Cooke, causal agent of tomato leaf mold, and the genetics of resistance to it began as a result of a suggestion by Dr. J. W. MacArthur of the Department of Zoology, University of Toronto, that the linkage relations of a recently discovered factor governing immunity from *C. fulvum* be determined. Dr. MacArthur provided hybrid seeds for the project, which, it was estimated, would take one summer to complete. The early discovery of additional types of resistance and of physiologic races of *C. fulvum* broadened the problem and its strictly phytopathological aspects assumed such importance that it became

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Joint contribution from Bishop's University, Lennoxville, Que., the University of Toronto, Toronto, Ont., and the Vineland Horticultural Experiment Station, Vineland Station, Ont.

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acceptable as a thesis project in the Department of Botany, University of Toronto, where the author was working under the direction of Dr. D. L. Bailey. In this manner there originated a co-operative project that has led to several lines of investigation that could not be foreseen at the time. The results of some genetical and pathological aspects of this project have been published (15, 16). It may be recalled here that the factor  $Cf_p$ , found in the red currant tomato, *Lycopersicon pimpinellifolium* (Jusl.) Mill., enables the development of immunity from *Cladosporium fulvum* physiologic races 1 to 4, and that its recessive allele,  $cf_p$ , apparently characteristic of all varieties of the ordinary cultivated tomato, *L. esculentum* Mill., permits (other resistance factors being absent) the development of plants completely susceptible to these physiologic races.

Previous to 1936, efforts to produce a tomato variety immune from *C. fulvum* occupied a minor place in the entire problem. So far as the author is aware von Sengbusch and Loschakowa-Hasenbusch (20) were the first investigators to realize the possibility of developing large-fruited esculentum-type varieties immune from *C. fulvum*. This possibility was also considered by Dr. MacArthur, who had made independent observations of the inheritance of immunity from *C. fulvum*. Dr. MacArthur suggested that immune segregants from crosses between *L. esculentum* and *L. pimpinellifolium* be crossed with large-fruited field varieties of the Earliana type, with the object of achieving large fruit size rapidly. Since it was desired to produce a variety particularly suited to our greenhouse conditions, and since the factor for immunity was known to be dominant, it seemed that it would be simpler and more satisfactory to secure the desired type by successive backcrosses of immune segregants to the English variety Potentate, which was recommended by the Horticultural Experiment Station at Vineland Station, Ont. as giving excellent performance in the greenhouse under local conditions. This procedure would obviate the necessity of eliminating by selection certain undesirable features of field tomatoes such as the frequently encountered inability to set fruit readily under greenhouse conditions. Eight successive backcrosses to Potentate would produce a line with a residue of approximately 1/256th *L. pimpinellifolium* inheritance, and it was believed that such a line would be essentially like an 'immune Potentate'. This breeding plan was adopted.

The production of the variety later named Vetomold began with a cross between Potentate and an immune  $F_2$  segregant from a cross between *L. pimpinellifolium* and Dr. MacArthur's esculentum selection No. 705. In the first few generations only 10 to 15 plants per crop were used for this phase of the work. Successive backcrosses resulted each generation in the production of plants more esculentum-like in appearance and with greater fruit size. By the summer of 1935, however, it was evident that there might be some difficulty in establishing a line in which there did not occur a high proportion of seriously abnormal individuals. These individuals, until then found in all populations segregating with respect to immunity and susceptibility, were characterized by the appearance, after several weeks of apparently normal

development, of a spontaneous and progressive 'necrosis' of the leaves (Figs. 1 to 4). At this point the breeding program was expanded greatly and the problem of developing a variety immune from *C. fulvum* became largely one of investigating the nature of 'necrosis' and of attempting to establish lines free from this condition. The introduction of the variety Vetomold in 1939 followed the elucidation of the nature of necrosis and the establishment of immune, necrosis-free lines horticulturally very similar to the variety Potentate.

Alexander, in Ohio, and Guba, in Massachusetts, were also at work during this period incorporating the immunity of *L. pimpinellifolium* in varieties of tomato suited to their local conditions and local markets. They encountered a condition that is apparently the same as our necrosis. In 1938 Alexander (1), in reporting upon the production of the variety Globelle from a cross between *L. esculentum* var. Globe and *L. pimpinellifolium*, stated: "What appeared to be a factor for a partial lethal was observed in the  $F_2$  generation of the original cross. Subsequent observations indicate that this factor is linked with the  $Cf_{p_1}$  resistant factor." In 1942 Guba (12), discussing the development of the variety Bay State from a cross between *L. pimpinellifolium* and *L. esculentum* var. Waltham Forcing, wrote "In the fall of 1939, a larger  $F_3$  population was grown in several greenhouses. The performance and quality of the tomato were unusually promising but there were also some inherently bad features, notably an hereditary breakdown, manifested by yellowing, rusting, spotting, and necrosis of the foliage."

In 1942 the author (16) made a preliminary report describing briefly the nature and symptoms of necrosis and characterizing the new variety Vetomold as essentially the esculentum variety Potentate except for two dominant factors derived from *L. pimpinellifolium*:  $Cf_{p_1}$ , the immunity factor, and  $Ne$ , the factor preventing the development of necrosis. The factor  $Ne$  was located on Chromosome I.

In 1945 Clarke and Sherrard (8) discussed the appearance of symptoms of what is undoubtedly the condition described as necrosis in our studies. Necrosis appeared in their  $F_2$  generations from crosses between Vetomold and various esculentum varieties. Clarke and Sherrard report the behavior of small populations extending to the  $F_4$  generation, and state that their data seem to "suggest that the trouble is genetical in nature, leaf spot behaving as a recessive character".

The present belated paper constitutes a more extensive report on the nature and behavior of necrosis.

### Materials and Methods

Conidia of *C. fulvum* for inoculation purposes were secured almost exclusively from infected tomato leaves from various greenhouses in the Niagara peninsula, Ontario. Previous to 1940 no attempt was made to determine which physiologic races of the fungus were present in the collections, since the factor  $Cf_{p_1}$  conferred immunity from all four physiologic races of the fungus then recognized. In the autumn of 1939, following the introduction of the

variety Vetomold, there was discovered an additional strain of *C. fulvum* capable of infecting Vetomold and other 'immune' hybrids. It thus became necessary, in connection with the necrosis problem, to obtain conidia from certain greenhouses in which the 'new' strain of the fungus had not yet appeared, as evidenced by the freedom of Vetomold from disease in the presence of a serious epiphytotic on other varieties. Nevertheless, because of the concentration of the 'new' strain built up in the Vineland greenhouses in 1940, it became increasingly difficult, during the summer months, to classify populations segregating for susceptibility to and immunity from races 1 to 4. Heavy conidial suspensions were made up in tap water just before they were to be used and both upper and lower surfaces of the leaves were heavily sprayed by means of an electrically powered DeVilbiss atomizer. After the foliage of the plants had dried the plants were transferred to a factory cotton inoculation chamber placed directly over wet soil in a greenhouse bed. Inoculations were usually carried out in the late afternoon or evening: the chamber was thoroughly wetted then and at intervals thereafter: the next afternoon the chamber was allowed to dry gradually and the plants were usually removed from it by the evening of the same day.

In preparation for the fall greenhouse crop the seeds were sown individually at the rate of 200 per seed flat (about  $18 \times 12$  in. in size), thus allowing the seedlings to become large enough for inoculations while still in the flats, with consequent saving of space and labor. In July the usual age of the seedlings at the time of inoculation was about 16 days. Using the method outlined, 2000 or more plants could be handled at one time in the inoculation chamber. The seedlings were usually planted in pots the day after the inoculation was completed. This schedule enabled the completion of pathogenicity tests before it was time to set the plants in the soil of the field or greenhouse.

During midwinter, when the author was at the University of Toronto, or at Bishop's University, the procedure just described was found to be impracticable. The spring crop was planted before the performance of any test of immunity; crosses were made on apparently normal plants and, in the spring, after the induction of an epiphytotic, the non-necrotic plants were classified as immune or susceptible and the selection of useful crosses made upon the basis of this classification. The epiphytotic was induced in a variety of ways such as late opening and early closing of the greenhouse vents to increase the relative humidity, excessive watering, and the broadcasting of a heavy suspension of conidia by means of an electrically powered atomizer.

Unless otherwise noted all experiments were conducted at Vineland Station, Ont., latitude N.  $43^{\circ} 13'$ , longitude W.  $79^{\circ} 23'$ .

Immer's (14) tables were used for the calculation of recombination values in the linkage experiments but standard errors are used throughout the paper. The  $\chi^2$  values given are unadjusted unless otherwise stated.

Throughout the paper reference is made to various pairs of characters in the tomato by symbol only. The symbols for the recessive members of the

corresponding factor pairs, together with the characters whose development they condition, are listed at this point for reference purposes.

$a_1$ —green stem	$o$ —elongated fruit
$c$ —potato leaf	$p$ —peach (hairy) fruit surface
$cf_{p_1}$ —susceptibility to <i>C. fulvum</i> races 1 to 4	$r$ —yellow fruit flesh
$d_1$ —dwarf stature	$s$ —compound inflorescence
$f$ —fasciated fruit shape	$t$ —tangerine orange fruit flesh
$h$ —hairy stem	$u$ —uniformly colored unripe fruits
$j$ —jointless pedicel	$wt$ —wilty foliage
$lf$ —leafy inflorescence	$y$ —clear (non-yellow) fruit epicarp

The factor  $cf_{p_1}$  is located on Chromosome III. Table VII shows the location of the other factors. Further information concerning these factors and characters is available in articles by MacArthur (17); Butler (6), for  $j$  only; Langford (15), for  $cf_{p_1}$  only.

In the present studies the lineage of all plants immune from *C. fulvum* may be traced to a single sample of seed of *L. pimpinellifolium* purchased from Vaughan's Seed Store, Chicago, Ill., in 1922, under the designation 'Red Currant Tomato'.\* The appearance of plants of this strain of the red currant tomato is in keeping with Muller's (19) description of *L. pimpinellifolium*.

### Experimental Results

The condition here described and characterized as 'necrosis' has developed among plants that are derivatives of crosses between the ordinary cultivated tomato, *L. esculentum*, and the red currant tomato, *L. pimpinellifolium*, and retain the factor  $Cf_{p_1}$  in whose presence a plant may develop immunity\*\* from *C. fulvum* physiologic races 1 to 4. This necrotic condition has not been observed in any other situation.

### SYMPTOMATOLOGY OF NECROSIS

There is considerable variation in the expression of necrosis. Although the greatest development of the condition may be seen in a crop of tomatoes grown under glass in midsummer the picture presented by an early autumn crop of tomatoes is described to show the onset of symptoms of necrosis. A summer greenhouse crop is seldom raised and plants of a spring crop comparable in degree of maturity with those of an early fall crop that is showing necrosis are themselves typically free from symptoms of necrosis. The age at which symptoms of necrosis appear in a plant varies but under our conditions plants from seed sown during the first week of July, although normal in appearance at the age of one month when grown directly in the soil under commercial conditions, typically have developed symptoms of necrosis by

\* Vaughan's have no information concerning the origin of this seed lot.

\*\* NOTE—Hereafter immunity as used in this paper refers to immunity from *Cladosporium fulvum* physiologic races 1 to 4.

the age of six weeks to such an extent that a population may be classified readily. In such plants the first clearly recognizable symptom of the abnormality is the occurrence of small, angular, yellowish to brown, necrotic spots near the tip of one or more of the lower leaves of the plant (Fig. 1). Such spots are much more apparent on the lower than on the upper surface of the leaves. The spots are not superficial and a single spot may be viewed from either the lower or the upper surface of the leaf. Accompanying the spotting is a downward and inward cupping of the leaf, essentially like that of the leaves of the potted plant shown in Figs. 2 and 3. Later symptoms consist largely of an intensification of those just described (Fig. 4). The curling of the leaf becomes more pronounced and all the leaflets typically draw together beneath the rachis. The necrotic spots become larger and more numerous, with coalescence, and plainly visible from the upper surface of the leaf as well as from the lower one. If the environmental conditions remain suitable for the development of necrosis the changes described continue, progressively, from the tip of a leaflet to its base and from distal to proximal leaflets (Fig. 3). The symptoms follow the growth of the plant upward, younger and still younger leaves successively developing the symptoms. At any time, however, the youngest growth typically remains normal in appearance (Fig. 2). Laterals that are allowed to develop are at first normal in appearance but later develop symptoms in the same sequence as does the foliage associated with the main stem. In the autumn conditions do not remain suitable for the typical development of necrosis, thus the later symptoms described are those that would be seen in the latter part of a spring crop or in a summer crop.

A plant in which severe necrosis has developed bears fruit of greatly reduced size. Under conditions of prolonged intense sunlight the affected plants may be completely browned except near the growing point, their leaves largely shrivelled and dead.

In the case of moderate and severe necrosis (as judged by the amount of the foliage killed), and sometimes in cases of slight necrosis, characteristic pedicel symptoms develop, especially if the truss has been well exposed to the light. The cortical tissue is necrotic: sometimes there are a few bits of green tissue but often the necrosis is so extensive that the whole truss presents a brown, killed appearance. In very severe cases the main stem may also become necrotic and in some cases necrosis is undoubtedly a factor contributing to the death of a plant in the field. In the greenhouse there has been no suggestion of an association between the presence of necrosis and the death of a plant.

#### THE AUTOGENOUS NATURE OF NECROSIS

Although, early in the investigation, it seemed that necrosis was of non-parasitic origin, it was considered desirable to test its supposedly autogenous nature.

Pieces of tissue including necrotic spots were cut from tomato leaves and surface sterilized with a 1 : 1000 aqueous solution of mercuric chloride and plated on potato dextrose agar and on malt agar. This procedure did not



*Symptoms of necrosis on tomato plants of genotype  $Cf_{v1}/Cf_{v1}; ne/ne$ .*

FIG. 1. Early symptoms. Necrotic spots on upper surface of a leaflet (nine-weeks-old plant from a commercial planting, photographed Sept. 10).

FIG. 2. Symptoms on fifth and younger leaves of a one-month-old plant grown in a 4-in. pot (photographed Aug. 8).

FIG. 3. Downward and inward cupping of sixth leaf of plant photographed in Fig. 2.

FIG. 4. Later symptoms. Coalescence of necrotic spots and shrivelling of leaflets (nine-weeks-old plant from a commercial planting, photographed Sept. 10).

Fig. 2,  $\times 0.2$  approximately; other Figs.  $\times 0.6$ , approximately

Photographs by T. Armstrong, Dominion Entomological Laboratory, Vineland Station, Ont.





reveal any organism causally or even consistently associated with the necrotic spots. Microscopic observations of similar pieces of leaf tissue cleared in lactophenol mixture and stained with cotton blue (aniline blue) failed to show any trace of fungous mycelium in connection with the necrotic tissue.

The development of necrosis in many tomato crops indicated that it was not caused by a transmissible agent such as a virus that might be spread by pruning operations or through the activities of insects. Although symptoms of necrosis do not appear simultaneously on all potentially necrotic individuals of any one age, symptoms first appear on the plants during a reasonably short period, varying according to the season. Plants that have remained normal until necrosis has become serious on sister plants have not developed symptoms of necrosis subsequently in a single instance.

As a final check on the autogenous nature of necrosis a series of graftings and buddings was made, using combinations of necrotic and non-necrotic plants. Plants aged two and one-half months were used for this purpose on July 2. Successful combinations were made as follows, the stock being mentioned second in each instance:

Immune non-necrotic/necrotic	—two grafts and one bud
Susceptible non-necrotic/necrotic	—four grafts and one bud
Necrotic/susceptible non-necrotic	—two grafts and one bud
Necrotic/immune non-necrotic	—two grafts.

In all cases, some weeks after the union was well established, laterals from below the graft or bud level were allowed to grow. In no case was there observed any modification of the intrinsic expression of either partner of a union, with respect to the development of necrosis. Necrosis developed severely on the foliage of all potentially necrotic shoots, as on entire, sister, check plants grown at the same time in the same greenhouse bed.

## THE GENETICS OF NECROSIS

### THE MENDELIAN NATURE OF THE INHERITANCE OF NECROSIS

It has been pointed out above that necrosis develops only among certain plants possessing the immunity factor  $Cf_{D_1}$ . The data in this section show that the primary difference between immune, non-necrotic plants and immune plants having the ability to develop necrosis is due to a difference in genotype at one locus, designated as the  $ne$  locus. Non-necrotic plants are of the genotypes  $Ne/Ne$  and  $Ne/ne$ , homozygotes and heterozygotes being indistinguishable phenotypically; necrotic plants are of the genotype  $ne/ne$ . The theoretical ratios expected from various crosses, on the basis of this mode of inheritance of necrosis, are set forth in Table I. Unusual deviations from theoretical ratios and the influence of environmental factors on the development of potentially necrotic individuals are considered in later sections of this paper. The data presented in this section were obtained from populations grown under environmental conditions favoring the development of typical necrosis and in the absence of genetic factors that indicate that the

TABLE I

THE INHERITANCE OF NECROSIS IN POPULATIONS DERIVED FROM CROSSES BETWEEN  
*Lycopersicon pimpinellifolium* AND *L. esculentum*

Plant types crossed	Plant type numbers, plant characterizations, and genotypes of parents and progeny, with theoretical ratios of progeny				
	1. <i>L. pimpinellifolium</i> <i>Cf<sub>p1</sub>/Cf<sub>p1</sub>; Ne/Ne</i> immune, non-necrotic		2. <i>L. esculentum</i> <i>cf<sub>p1</sub>/cf<sub>p1</sub>; ne/ne</i> susceptible, non-necrotic		
1 × 2 or 2 × 1	All <i>Cf<sub>p1</sub>/cf<sub>p1</sub>; Ne/ne</i> immune, non-necrotic				
	3. <i>F<sub>1</sub></i> of cross 1 × 2 or 2 × 1 or any plant of same genotype, derived from later backcrosses or pseudo-back- crosses <i>Cf<sub>p1</sub>/cf<sub>p1</sub>; Ne/ne</i> immune, non-necrotic		4. <i>L. esculentum</i> <i>cf<sub>p1</sub>/cf<sub>p1</sub>; ne/ne</i> susceptible, non-necrotic		
3 × 4 or 4 × 3	1 <i>Cf<sub>p1</sub>, Ne</i>	: 1 <i>Cf<sub>p1</sub>, ne</i>	: 1 <i>cf<sub>p1</sub>, Ne</i>	: 1 <i>cf<sub>p1</sub>, ne</i>	
	1 immune, non-necrotic	: 1 immune, necrotic	:	2 susceptible, non-necrotic	
	5. <i>Cf<sub>p1</sub>/cf<sub>p1</sub>; Ne/ne</i> any immune, non-necrotic segregant from a cross of type 3 × 4 or 4 × 3				
5 selfed or 5 × 5	9 <i>Cf<sub>p1</sub>, Ne</i>	: 3 <i>Cf<sub>p1</sub>, ne</i>	: 3 <i>cf<sub>p1</sub>, Ne</i>	: 1 <i>cf<sub>p1</sub>, ne</i>	
	9 immune non-necrotic	: 3 immune, necrotic	:	4 susceptible, non-necrotic	
Selfing of immune non- necrotic plants from 5 selfed or 5 × 5	Genotypes and ratios of genotypes among immune, non-necrotic parents		Theoretical ratios of progeny from the four parental genotypes		
			Immune, non-necrotic	Immune, necrotic	Susceptible, non-necrotic
	1 <i>Cf<sub>p1</sub>/Cf<sub>p1</sub>; Ne/Ne</i>		1	0	0
	2 <i>Cf<sub>p1</sub>/Cf<sub>p1</sub>; Ne/ne</i>		3	1	0
	2 <i>Cf<sub>p1</sub>/cf<sub>p1</sub>; Ne/Ne</i>		3	0	1
	4 <i>Cf<sub>p1</sub>/cf<sub>p1</sub>; Ne/ne</i>		9	3	4

simple Mendelian interpretation of the inheritance of necrosis is incomplete. The pertinent data, selected after the examination of well over 10,000 plants of segregating populations grown to maturity in the field and in the greenhouse, follow, in six parts.

### 1. The Occurrence of Necrosis as Related to Immunity from *Cladosporium*

Necrosis has never developed in a plant of genotype  $cf_{p1}/cf_{p1}$ , i.e. a plant lacking immunity from *Cladosporium*, but may develop in plants of genotype  $Cf_{p1}/Cf_{p1}$  or  $Cf_{p1}/cf_{p1}$ .

### 2. Backcrosses and Pseudobackcrosses

When immune necrotic plants derived from crosses with esculentum varieties are crossed with susceptible, esculentum-type varieties, the progeny segregates in the ratio of approximately 1 immune non-necrotic : 1 immune necrotic : 2 susceptible non-necrotic. See Table I, Cross 3  $\times$  4, and Table II. Although Table II is based on examination of 66 populations, these were in many cases so alike in derivation that they were grouped into the 28 classes shown. Chi<sup>2</sup> tests of heterogeneity indicate the appropriateness of combining the populations in this way. The approximate values of  $P$  corresponding to the values of  $\chi^2$  calculated for the 17 groups of populations (3  $F_2$ 4P, 2 5GR, 6  $F_2$ 3P<sup>a</sup>2P, . . . . . to 2 2P of the 1939 crop) are 0.5, 0.2, 0.7, 0.9, 0.05, 0.8, 1.0, 0.5, 0.04, 0.5, 0.3, 0.4, 0.2, 0.7, 1.0, 0.2, and 0.9, respectively. In the upper half of the table it may be seen that the pooled ratio from 14 groups of populations agrees very closely with the theoretical ratio of 1 : 1 : 2, but that the homogeneity of the populations is not very great. It will be noted, however, that the 5GR group of 1937 is the only group that does not give a satisfactory fit to a 1 : 1 : 2 ratio and also that the poor fit is due to departure from the expected ratio of 1 immune : 1 susceptible and not from the expected ratio of 1 normal : 1 necrotic. Inasmuch as the monofactorial inheritance of immunity from *Cladosporium* has been established and verified (20, 15) this deviation is not considered to be an important one. If this group is eliminated from the calculation the remaining 13 groups yield a pooled ratio of 168 : 166 : 350, with a  $\chi^2$  value of 0.385 and a  $P$  value of 0.8. The heterogeneity  $\chi^2$  thus becomes  $26.172 - 9.056 + 0.385 = 17.501$ , with a corresponding  $P$  value of 0.1, indicating fairly satisfactory homogeneity.

In the last two columns of Table II  $\chi^2$  and  $P$  values for the immune plants only test the approach of the observed ratios to 1 : 1. The agreement with a theoretical 1 : 1 ratio is very close indeed. Two small populations deviate beyond the 5% level but since 28 populations are represented in the table the general fit is considered satisfactory. The addition of the immune plants of the upper and lower parts of Table II gives 504 normal and 513 necrotic, a  $\chi^2$  value of 0.080 and a  $P$  value of approximately 0.8. The heterogeneity  $\chi^2$  becomes 26.343 and  $P$ , with 27 degrees of freedom for the  $\chi^2$  value, approximately 0.5. It is thus clearly shown that the expectations from crosses of the type 3  $\times$  4 in Table I have been realized satisfactorily, particularly with regard to the segregation of factors at the *ne* locus.

### 3. The Selfing of Heterozygotes

Immune non-necrotic hybrids, themselves resulting from crosses with susceptible esculentum varieties, yielded, on selfing or intercrossing, plants in the ratio of approximately 9 immune non-necrotic : 3 immune necrotic : 4

TABLE II

THE INHERITANCE OF NECROSIS IN POPULATIONS DERIVED FROM CROSSES BETWEEN  
HETEROZYGOUS IMMUNE, NON-NECROTIC TOMATOES AND SUSCEPTIBLE  
ESCULENTUM VARIETIES. SEE TABLE I, CROSS 3 X 4

Year and crop <sup>a</sup>	No. of populations and population designations <sup>b</sup>	n	Segregation ratios			$\chi^2$ (1 : 1 : 2)	P (approx.)	$\chi^2$ (1 : 1) immune plants only	P (approx.)
			Immune, non-necrotic	Immune, necrotic	Susc., non-necrotic				
1935, summer	1 F <sub>3</sub> 3P	18	6	4	8	1.111	0.6	0.400	0.5
1936, spring	1 2P	45	13	12	20	0.599	0.7	0.040	0.8
	1 3GR	18	6	4	8	1.111	0.6	0.400	0.5
	3 F <sub>4</sub> 4P	99	26	27	46	0.515	0.8	0.019	0.9
1937, spring	1 4P	9	3	1	5	1.000	0.6	1.000	0.3
	2 5GR	27	9	12	6	9.056	<0.01 <sup>c</sup>	0.429	0.5
	6 F <sub>3</sub> 3P*2P	153	36	36	81	0.529	0.8	0.000	1.0
	3 F <sub>2</sub> 6P	108	20	25	63	3.462	0.2	0.555	0.5
1938, spring	2 F <sub>3</sub> 3P*2P	36	9	12	15	1.500	0.5	0.429	0.5
	2 6P	36	11	5	20	2.445	0.3	2.250	0.1
	2 7GR	36	6	10	20	1.333	0.5	1.000	0.3
	5 F <sub>2</sub> 8P	90	25	21	44	0.400	0.8	0.348	0.5
1939, spring	1 8P	18	4	6	8	1.111	0.6	0.400	0.5
	1 F <sub>1</sub> 10P	18	3	3	12	2.000	0.4	0.000	1.0
						26.172		7.270	
		711	177	178	356	0.054	0.98	0.003	0.95
			Heterogeneity $\chi^2$ (13 degrees of freedom)			26.118	0.02 <sup>c</sup>	7.267	0.9
1937, fall	1 5P	18	7	11	(Susceptible plants from these populations were not grown to maturity, and the behavior of mature plants only is here reported)			0.889	0.35
	2 6GR	32	14	18				0.500	0.5
	5 F <sub>3</sub> 3P*3P	80	34	46				1.800	0.2
	5 F <sub>7</sub> 7P	102	57	45				1.411	0.2
1938, field	2 2P	16	12	4	(Susceptible plants from these populations were not grown to maturity, and the behavior of mature plants only is here reported)			4.000	0.05 <sup>c</sup>
	1 3P	13	6	7				0.077	0.8
	1 4P	17	5	12				2.884	0.1
	1 5P	18	7	11				0.889	0.35
	3 6P	43	22	21				0.023	0.9
1938, fall	5 F <sub>3</sub> 3P*P*2P	77	40	37	(Susceptible plants from these populations were not grown to maturity, and the behavior of mature plants only is here reported)			0.117	0.7
	2 7P	36	16	20				0.444	0.5
	4 F <sub>2</sub> 9P	64	33	31				0.063	0.8
1939, field	2 2P	128	60	68	(Susceptible plants from these populations were not grown to maturity, and the behavior of mature plants only is here reported)			0.500	0.5
1939, fall	1 8P	18	14	4				5.556	0.02 <sup>c</sup>
								19.153	
		662	327	335				0.097	0.8
			Heterogeneity $\chi^2$ (13 degrees of freedom)					19.056	0.1

<sup>a</sup> Spring, summer, and fall, of this column, refer to crops grown under glass.

<sup>b</sup> All populations are descended from crosses between *L. pimpinellifolium* and *L. esculentum*. The symbols P and GR refer to the esculentum varieties Potentate and Grand Rapids and the numerals preceding them indicate the number of times these varieties have been crossed into the line. Superscripts, \*, \*\*, etc., indicate 1, 2, etc. generations of selfing. The symbol F<sub>n</sub> refers to a single immune F<sub>n</sub> segregant from a cross between *L. pimpinellifolium* and *L. esculentum*.

<sup>c</sup> Values of P less than 0.05.

susceptible non-necrotic. See Table I, cross  $5 \times 5$  or 5 selfed, and Table III. The ratios obtained agree reasonably well with expectation.

TABLE III

THE INHERITANCE OF NECROSIS IN POPULATIONS DERIVED FROM SELFING OR INTERCROSSING IMMUNE, NON-NECROTIC TOMATO HYBRIDS, THEMSELVES RESULTING FROM CROSSES WITH SUSCEPTIBLE VARIETIES OF *L. esculentum*. SEE TABLE I, CROSS  $5 \times 5$  AND 5 SELFED (NOTE. FOOTNOTES <sup>a</sup>, <sup>b</sup>, <sup>c</sup>, OF TABLE II ALSO APPLY TO THIS TABLE)

Year and crop	No. of populations and population designations	n	Segregation ratios			$\chi^2$ (9 : 3 : 4)	P (approx.)
			Immune, non-necrotic	Immune, necrotic	Susceptible, non-necrotic		
1936, spring	2 $F_3P^a$	177	102	27	48	1.532	0.5
1937, spring	1 $F_3P^aP^a$	17	10	2	5	0.596	0.7
1938, field	30 $F_4P^a$	1689	969	304	416	0.976	0.6
						3.104	
		1883	1081	333	469	1.598	0.5
		Heterogeneity $\chi^2$ (2 degrees of freedom)				1.506	0.5

The bottom section of Table I shows the four different genotypes that may be expected among the approximately 9/16th immune non-necrotic individuals of populations such as those recorded in Table III. Further selfing of plants of one of these genotypes, and one only,  $Cf_{p_1}/cf_{p_1}$ ;  $Ne/ne$ , yields the phenotypic ratio 9 immune non-necrotic : 3 immune necrotic : 4 susceptible non-necrotic. Table IV includes data from all populations, including those of Table III, segregating into these three classes, regardless of their origin. The numbers of susceptible plants are not included in this table because we are primarily concerned with the  $Ne-ne$  factor pair and also because in many instances all of the susceptible plants of a population and some of the immune plants were discarded from 4 in. pots after the reading of their reaction to *Cladosporium*; thus, with no test of the occurrence of necrosis among the surplus immune plants, the inclusion of a figure for susceptible plants would have required the listing of fractions of plants, complicating the calculations and making no essential contribution to the analysis. Table IV shows satisfactory homogeneity of the populations grouped and of the groups of populations: the ratios of non-necrotic to necrotic plants agree well with expectation. It will be noted that nearly all of the departure from a 3 : 1 ratio in the final pooled ratio is accounted for by population 13. In view of the linkage of  $ne$  and  $d_1$ , part of this deficiency in necrotic individuals is believed to be referable to the shortage of about 10% from expectation among the dwarf class ( $d_1$ ) in this population. See Table VII.

TABLE IV

THE INHERITANCE OF NECROSIS IN POPULATIONS DERIVED FROM THE SELFING OF HETEROZYGOUS IMMUNE, HETEROZYGOUS NON-NECROTIC TOMATO HYBRIDS,  $Cf_{P1}/cf_{P1}$ ;  $Ne/ne$ , DERIVED FROM VARIOUS SOURCES (NOTE. FOOTNOTES <sup>a</sup>, <sup>b</sup>, <sup>c</sup> OF TABLE II ALSO APPLY TO THIS TABLE)

Population or population group number, year, and crop	Number of populations and population designations	Heterogeneity tests of grouped populations		Number of immune plants			$\chi^2$ (3 : 1)	P (approx.)
		$\chi^2$	P (approx.)	n	Non-necrotic	Necrotic		
1, 1936, spring	2 $F_2P^a$	0.158	0.7	129	102	27	1.140	0.3
2, 1937, spring	1 $F_2P^aP^a$			12	10	2	0.444	0.5
3, 1938, spring	1 $F_2P^aP^aP^a$			23	21	2	3.260	0.08
4, 1938, spring	5 $F_2P^a$	5.633	0.2	109	85	24	0.517	0.5
5, 1938, field	30 $F_2P^a$	15.724	0.98	1273	969	304	0.851	0.4
6, 1938, field	31 $F_2P^a$	21.666	0.85	1309	954	355	3.137	0.08
7, 1939, spring	2 7GR <sup>a</sup>	1.028	0.3	30	24	6	0.400	0.5
8, 1937, fall	6 $F_2P^aP^aP^a$	6.847	0.25	98	73	25	0.014	0.9
9, 1937, fall	7 $F_2P^a$	7.745	0.25	109	80	29	0.150	0.7
10, 1938, fall	8 $F_2P^aP^aP^aP^a$	9.627	0.2	203	149	54	0.278	0.6
11, 1938, fall	3 $F_2P^a$	0.753	0.7	86	63	23	0.140	0.7
12, 1938, fall	1 7GR <sup>a</sup>			18	17	1	3.631	0.06
13, 1939, field	1 $F_2$ of ( $F_2P^a$ $\times L. esculentum$ )			1096	850	246	3.815	0.05
14, 1939, fall	1 7GR			18	15	3	0.667	0.4
15, 1940, field	1 $F_2$ of ( $F_2P^a$ $\times F_2P^a$ )			106	82	24	0.314	0.6
							18.758	
							1.022	0.3
							17.736	0.2
							Heterogeneity $\chi^2$ (14 degrees of freedom)	

Figures on the numbers of susceptible plants are available not only for Populations 1, 2, and 5 of Table IV, as shown in Table III, but for Populations 3, 4, 6, and 7. The pooled ratio for these seven populations is 2165 immune non-necrotic : 720 immune necrotic : 954 susceptible non-necrotic. The  $\chi^2$  (9 : 3 : 4) value is 0.054 and the probability value,  $P$ , is 0.98. These figures fit the theoretical ratio very closely.

#### 4. Second Generation Selfing

Selfing of the immune non-necrotic individuals of populations arising from the selfing of plants presumed to be of the genotype  $Cf_{P1}/cf_{P1}$ ;  $Ne/ne$  yielded

TABLE V

THE INHERITANCE OF NECROSIS IN 74 POPULATIONS DERIVED FROM SELFING THE IMMUNE, NON-NECROTIC INDIVIDUALS IN POPULATIONS FROM THE SELFING OF TOMATO PLANTS OF THE GENOTYPE  $Cf_{p_1}/cf_{p_1}; Ne/ne$ . SEE ALSO THE BOTTOM SECTION OF TABLE I

Class of population	Segregation ratios expected			No. of populations with approximately the ratios shown		$\chi^2$ (1 : 2 : 2 : 4)
	Immune, non-necrotic	Immune, necrotic	Susceptible, non-necrotic	Expected	Observed	
A	1	0	0	8.20	9	0.078
B	3	1	0	16.45	13	0.724
C	3	0	1	16.45	21	1.259
D	9	3	4	32.90	31	0.110

$$\chi^2 \text{ (3 degrees of freedom)} = 2.171$$

$$P = 0.5$$

results in accordance with the expectations shown in the bottom section of Table I. These results are set forth in Table V. The pooled ratios among the 13, 21, and 31 populations of Classes B, C, and D, respectively, of Table V, agree well with the expectations shown at the left of the table, as follows:

Class B—563 immune non-necrotic : 190 immune necrotic ( $P = 0.8$ ).

Class C—912 immune non-necrotic : 1 immune necrotic (obviously a misplaced plant, and not included in the calculation) : 300 susceptible ( $P = 0.7$ ).

Class D—954 immune non-necrotic : 355 immune necrotic : 445 susceptible non-necrotic ( $P = 0.2$ ).

### 5. Breeding Behavior of Necrotic Plants

It is apparent that necrotic plants may be of the genotypes  $Cf_{p_1}/Cf_{p_1}; ne/ne$  and  $Cf_{p_1}/cf_{p_1}; ne/ne$ . Inasmuch as there was no interest in breeding a quantity of necrotic lines and since their behavior was so very obvious very little data were accumulated concerning them. However, as would be expected, the selfing of a necrotic heterozygous immune plant or the crossing of it to an esculentum variety yielded, in both instances, immune plants 100% of which were necrotic and susceptible plants that were non-necrotic. A few races of homozygous immune necrotic types have been established and inbreeding of such races has produced immune necrotic plants only.

### 6. Homozygous Immune Non-necrotic Lines

Any plant of a population whose members were all immune from *C. fulvum* and non-necrotic (populations of Class A, Table V) remained true to this type on inbreeding. Various lines of this type have been established, one of which is the variety Vetomold, introduced jointly in 1939 by the Ontario Horticultural Experiment Station and the University of Toronto. Vetomold is of the genotype  $Cf_{p_1}/Cf_{p_1}; Ne/Ne$ .



## ENVIRONMENTALLY INDUCED VARIATIONS IN THE EXPRESSION OF NECROSIS

The data presented in the preceding section, showing very clearly the simple, Mendelian nature of the inheritance of necrosis, were obtained from populations grown under conditions favoring the development of typical necrosis. The importance of the role of the environment in the development of necrosis has been shown repeatedly during the course of the investigation: a few illustrations follow.

Seasonal variations in the severity of necrosis have been very conspicuous. In general, the development of necrosis is most severe when greenhouse temperatures and the intensity of incident light are high. Plants of a line that develops severe necrosis during midsummer may appear normal during midwinter unless they are grown in a section of the greenhouse in which incident light intensity is unusually high. At Vineland Station the greenhouses are laid out on a north-south axis with large vertical exposures of glass at their southern ends. During midwinter genetically necrotic plants in the most southerly row have developed moderate to severe necrosis while similar plants in the center of the same house could not, with certainty, be identified as necrotic. In a spring crop of tomatoes, from seed sown between late December and mid-January, symptoms of necrosis are delayed greatly but appear with the coming of bright spring weather and higher temperatures, and at maturity the necrotic plants are seriously impaired functionally, producing a light yield and presenting, ultimately, a generally brown appearance. In a fall crop, from seed sown about July 11, the first symptoms of necrosis may develop characteristically by the age of about six weeks but usually do not become serious and after September new growth remains practically normal in appearance. Under these conditions the yield from genetically necrotic plants is not significantly lowered.

The time of sowing seed for the fall crop has been found to be critical with respect to the development of symptoms of necrosis. For many years July 11 has been the normal date for sowing seed for the fall crop at Vineland Station. Advancing this date a week or more has made the clear phenotypic segregation of necrotic and normal individuals a certainty whereas postponement of the sowing date for a week or more has resulted in the development of nearly normal plants that have proved difficult to classify.

Summer greenhouse crops have been grown year after year and in these the development of necrosis has been striking and severe, even in houses that had been coated with whitewash. The seriousness of the necrosis problem was, indeed, borne in to us through the observation of certain small greenhouse populations grown in the summer during the earlier stages of the development of the tomato leaf mold problem.

Typical necrosis developed in field plants of appropriate families. A population of 573 plants, for instance, from seed sown on May 31, 1940, was inoculated at age 17 days, and set out in the field at age 42 days, following a rainfall

of 1.16 in., unusual in this district. On Aug. 21, at age 82 days, necrosis, expected to appear in approximately half of the plants, was plainly visible and developing rapidly. Two days later a classification was made with ease, by simple and rapid examination of the gross appearance of the plants. A final, careful check two weeks later showed that, of the 266 necrotic individuals of this population, eight only had been missed in the Aug. 23 classification. The hours of sunshine for the months of June, July, and August were 230.9, 319.2, and 273.6, respectively, with total sunshine for the three months 6.3 hr. above the 31 year average: the monthly mean temperatures (averages of the daily means) for the same months were 63.1°, 70.5°, and 68.8° F., respectively, these averages being 1.8°, 0.8°, and 1.1°, respectively, below the 31 year average.

A second population from seed sown on June 24, 1940, and expected to yield a ratio of 1 non-necrotic : 1 necrotic, showed 360 non-necrotic and 221 necrotic plants,  $\chi^2$  being 33.26 and  $P$  far less than 0.01. This was a hybrid population segregating for various genetic factors, some of them linked with *ne*, and it is suspected that some of the deficiency in necrotic individuals is due to genetic causes. The data available are not sufficient to enable more accurate reference of this deficiency to environment and to heredity. However, data from this and other populations indicate that in the field as in the fall greenhouse crop, postponement of the sowing date results in the development of more nearly normal plants. Variations in the vigor of individual plants may account in part for the deficiency of necrotic individuals in a late field crop. It has been observed again and again that the greater the vigor of a plant the less the severity of necrosis. In connection with fall greenhouse crops surplus plants are often allowed to remain in 4 in. pots for some time after the crop has been planted. In these circumstances it has been noted that the less vigorous potted plants of a 100% necrotic line develop necrosis much earlier than do the sister plants set in the open soil, and the symptoms become more severe. The one-month-old plant photographed for Figs. 2 and 3, for instance, was grown in a 4 in. pot. It showed considerable development of necrosis, whereas the foliage of sister plants set in the soil of the greenhouse was still normal in appearance.

The effect of shading potentially necrotic individuals has been tested twice. Table VI summarizes the results of an experiment with a spring crop of tomatoes. The plants used were from an inbred line yielding 100% necrotic plants under suitable conditions. Seedlings were planted in 4 in. pots at age two weeks and, in a very vigorous, healthy condition, transplanted to 8 in. pots at age nine weeks, at which time 15 plants were moved from the open bench of greenhouse No. 1 to that of greenhouse No. 2. The shade of No. 2 was provided by a heavy, lattice-type blind. Two weeks later characteristic symptoms of necrosis were developing, particularly in the unshaded greenhouse No. 1. In greenhouse No. 2 two plants were symptomless and three practically free from necrosis; in No. 1 one plant was characterized as 'severely necrotic', its lowermost three leaves shrivelled and necrosis apparent to the

TABLE VI

THE EFFECT OF SHADING ON THE DEVELOPMENT OF NECROSIS IN POTENTIALLY NECROTIC TOMATO PLANTS GROWN FROM SEED SOWN AT TORONTO, ONT., ON JAN. 22, 1937

Greenhouse number and conditions under which the plants were grown after removal from locations A and B <sup>a</sup> at age 11 weeks	Number of plants selected from location		Symptoms of necrosis after 7½ weeks in new locations (Column 1)
	A	B	
No. 1, unshaded; open bench	4	1	All severe
No. 2, heavily shaded; open bench	4	3	Two slight, three moderate, two severe
No. 2, heavily shaded; plants grown in black broadcloth cage	4	3	Practically non-necrotic but plants weak and spindly
No. 3, unshaded; open bench	2	8	All severe

<sup>a</sup> Location A. Greenhouse No. 1, unshaded; open bench.

Location B. Greenhouse No. 2, heavily shaded on east side; open bench.

10th leaf. The other plants showed a considerable range of symptoms, the degrees of necrosis designated as from exceedingly slight to moderate. A representative plant showing 'slight necrosis' had 115 small necrotic spots on the third true leaf, fewer on leaf two, but none on leaf four. Six plants from greenhouse No. 1 were at approximately this stage of development, others showed more necrosis or less necrosis than these plants. Such variation in the speed of development of symptoms of necrosis has been observed repeatedly, even in lots which, like the family here used, had been treated as uniformly as possible. The disposition of these 11-week old plants is shown in columns 1 to 3 of Table VI. The black broadcloth cage to which some plants were transferred was about 6 ft. × 3½ ft. × 4 ft. high, open at the top, and placed on a gravel-covered bench in the center of the shaded greenhouse No. 2, in which incident light was reduced still further by running down a second heavy blind on the west side. Table VI shows clearly that reduction of light prevented the development of typical symptoms of necrosis. It may be added that the single plant earlier characterized as severely necrotic was placed in the cage and developed scarcely any symptoms of necrosis on its new foliage, whereas the two plants that were symptomless at the age of 11 weeks were placed in greenhouses No. 1 and No. 3 and both developed severe necrosis. Coincident with this experiment other segregating lines were grown in greenhouses Nos. 1 and 3. A typical, clear-cut segregation of non-necrotic and necrotic plants was evident in these populations.

The effect of shading on the development of necrosis in a fall crop was tested in 1939 in a commercial-type greenhouse at Vineland Station. Seed of a potentially necrotic line was sown on July 5 and the seedlings were planted in 4 in. pots on July 22, and protected from direct sunlight by a U-shaped formation of three seed flats placed on end. On Aug. 11 they were planted

in the most southerly row of the greenhouse and here protected from direct sunlight by a heavy shield of black broadcloth to the east, south, and west of them. Sister plants were planted in the same row, but exposed to full sunlight. On Aug. 26, by which time symptoms of necrosis were fairly general throughout the greenhouse, the four shaded plants, although noticeably spindly, showed no necrosis, while all of the other potentially necrotic plants in the same row showed unmistakable necrosis. The author soon returned to his winter work at Bishop's University and thus did not have the opportunity of watching these plants throughout the entire season. However, severe necrosis developed on the plants exposed to full sunlight, all except the uppermost leaves becoming brown and shrivelled whereas no such effects were observed on the shaded plants. That the symptoms on these exposed plants were not due simply to the high incidence of light on the plants of this most southerly row of the greenhouse, parallel to a large vertical plane of glass, was shown by the development of normal foliage on the individuals of non-necrotic lines interspersed in the row as checks.

These data show clearly that the expression of the factor combination  $Cf_{p_1}$ ,  $ne$  is very plastic: no further experiments have been undertaken to evaluate the roles of temperature and light in the development of symptoms of necrosis.

THE BEHAVIOR OF POPULATIONS FROM VARIOUS CROSSES BETWEEN *L. esculentum* AND *L. pimpinellifolium*

It seemed possible that necrosis might develop among the descendants of crosses between *L. pimpinellifolium* and some but not other varieties of *L. esculentum*. Accordingly crosses were made between *L. pimpinellifolium* and the widest variety of *esculentum* types conveniently available. The varieties used were Acme, Banalbufar, Beefheart, Bonny Best, Break O'Day, Crackerjack, Earliana, Early Detroit Purple, First of All, Grand Rapids, Imperial Globe, Potentate, Rouge Naine Hative, Sandblast, Tangerine, Tuckswood Favourite, and Wonder of Italy, most of these varieties being obtained from Dr. J. W. MacArthur's collection. In all instances the  $F_1$  generation plants were immune from *C. fulvum* and non-necrotic. In all cases the  $F_2$  populations showed a segregation of immune individuals into non-necrotic and necrotic individuals. In many instances the ratios gave a poor fit to a theoretical ratio of 3 normal : 1 necrotic among the immune plants (See Table IX and p. 55) but the data convinced us that necrosis would almost certainly be encountered among the descendants of any cross between *L. esculentum* and *L. pimpinellifolium*. In this connection the behavior of Dr. MacArthur's selection No. 32-13, a linkage-tester of genotype  $a_2$ ,  $d_1$ ,  $d_2$ ,  $f$ ,  $lf$ ,  $j$ ,  $br$ ,  $wt$ ,  $cf_{p_1}$ , is of interest. A very large  $F_2$  population from a cross of 32-13 selection with a selection of genotype  $Cf_{p_1}/Cf_{p_1}$ ;  $Ne/Ne$  was grown to test the linkage relations of the factor  $ne$ . Not a single necrotic plant appeared in this population, thus showing that selection 32-13 was of the genotype  $cf_{p_1}/cf_{p_1}$ ;  $Ne/Ne$ . Selection 32-13 traces back to a cross involving the variety

Burbank Preserving, which in turn comes from a cross between *L. pimpinellifolium* and *L. esculentum*: the gene *Ne* was accidentally retained in the selections resulting in the production of 32-13. Burbank Preserving is susceptible to *C. fulvum*; thus *Ne* was retained in the absence of *Cf<sub>p1</sub>*, which must be present if one wishes to test for the presence of *Ne*. This is the only unusual instance in which the factor *Ne* has been encountered.

#### THE LINKAGE RELATIONS OF THE NECROSIS FACTOR, *ne*

The linkage tests applied to the factor pair *Ne-ne* involved 16 pairs of contrasting characters. The difference between the members of each pair is controlled by a single pair of alleles. These factors and the corresponding characters are listed on p. 39. In each original cross all the dominant genes under consideration came from one parent and the recessives from the other. Apart from 93 *F<sub>2</sub>* plants from a cross between *L. pimpinellifolium* and *L. esculentum* and 99 plants from a backcross of such a cross to the esculentum parent, the inheritance of the plants furnishing linkage data was predominantly from *L. esculentum*.

Table VII summarizes the linkage relations of *ne*. The factors *d<sub>1</sub>*, *p*, *o*, and *s* have been located by MacArthur (17) on Chromosome I in the order stated. Our data show that *ne* is located on Chromosome I near *s* on the side distal from *d<sub>1</sub>*, *p*, and *o*. The author feels that the crossover value of 8.1% for *s-ne* may not be as close to the true value as the calculated standard error, 0.7%, suggests. Examination of the backcross data for *S-s* in Table VII reveals a ratio of 820 *S* : 553 *s* and a ratio of 883 *Ne* : 490 *ne*, in the face of expected ratios of 1 : 1 in each case. These deficiencies in the recessives might be caused by one or more of a number of factors, including (1) faulty breeding technique, (2) reduced viability of the recessives, (3) lack of suitable environmental conditions for the development of necrosis or of compound inflorescence in some plants, and (4) combinations of genes, other than *Ne*, that prevent an individual of genotype *ne/ne* from developing necrosis. If some seeds resulted from self-pollination rather than from a backcross to the double recessive decreased numbers of both *s* and *ne* individuals would be expected. Our experience with numerous other populations suggests, however, that this is not likely the explanation. The segregation ratio of 1407 *Cf<sub>p1</sub>* : 1326 *cf<sub>p1</sub>* ( $\chi^2 = 2.400$  and  $P = 0.12$ ) in this same population, although not as good a fit to a 1 : 1 ratio as is usually encountered, strengthens this view and indicates that little if any of the deviation in question is due to faulty breeding technique.

Data on the percentage of seed germination in this population were not kept but the behavior of other large populations, grown under environmental conditions favoring the development of necrosis, indicates that we are not dealing with a viability effect here.

The large population used to test linkage among *d<sub>1</sub>*, *p*, *o*, *s*, and *ne* was grown in three sections from seed sown on June 24, a little late for the best field development of necrosis at Vineland Station. Lot 1, of 426 plants, was grown in the greenhouse; Lot 2, of 600 plants, as a staked, field population; and Lot 3,

TABLE VII

LINKAGE RELATIONS OF *ne*, THE NECROSIS FACTOR FROM *L. esculentum*, BASED ON COUPLING DATA ONLY

Factor pair tested and number of chromosome bearing it	n	Segregation ratios <sup>a</sup>				$\chi^b$	Percentage recombination and standard error	Deviation divided by standard error
		<i>XY</i>	<i>Xy</i>	<i>xY</i>	<i>xy</i>			

<i>F<sub>2</sub></i> data										
<i>D<sub>1</sub></i>	<i>d<sub>1</sub></i>	I	1096	712	138	143	103	73.11	32.7 ± 1.8	9.6
<i>R</i>	<i>r</i>	II	934	560	173	149	52	0.44	48.3 ± 2.4	0.7
<i>Y</i>	<i>y</i>	III	2008	1095	416	380	117	3.05	53.0 ± 1.7	1.8
<i>C</i>	<i>c</i>	IV <sup>c</sup>	1096	675	175	196	50	0.01	50.2 ± 2.3	0.1
<i>A<sub>1</sub></i>	<i>a<sub>1</sub></i>	V	1096	650	200	182	64	0.65	48.1 ± 2.2	0.9
<i>J</i>	<i>j</i>	V	93	61	15	12	5	0.77	42.7 ± 7.1	1.0
<i>L<sub>f</sub></i>	<i>l<sub>f</sub></i>	V	93	61	15	12	5	0.77	42.7 ± 7.1	1.0
<i>L</i>	<i>l</i>	VI	1096	701	149	201	45	0.08	49.3 ± 2.2	0.3
<i>U</i>	<i>u</i>	VII	1074	587	191	234	62	1.55	52.9 ± 2.3	1.3
<i>H</i>	<i>h</i>	VII	1248	758	157	257	76	5.16	45.0 ± 2.0 <sup>d</sup>	2.5
<i>T</i>	<i>t</i>	VII	1074	580	198	220	76	0.01	49.8 ± 2.3	0.1
<i>Wt</i>	<i>wt</i>	X	93	55	21	12	5	0.02	48.8 ± 7.7	0.2

## Backcross data

<i>D<sub>1</sub></i> <i>d<sub>1</sub></i> I	1946	809	381	264	492	204.4	33.2 ± 1.1	15.3
<i>P</i> <i>p</i> I	987	389	209	109	280	129.2	32.2 ± 1.5	11.9
<i>O</i> <i>o</i> I	987	391	207	82	307	185.1	29.3 ± 1.4	14.8
<i>S</i> <i>s</i> I	1373	796	87	24	466	952.4	8.1 ± 0.7	59.9
<i>Y</i> <i>y</i> III	99	15	31	25	28	2.17	56.6 ± 5.0	1.3
<i>C</i> <i>c</i> IV <sup>c</sup>	573	146	161	130	136	0.10	50.8 ± 2.1	0.4
<i>A<sub>1</sub></i> <i>a<sub>1</sub></i> V	573	159	148	141	125	0.09	50.4 ± 2.1	0.2
<i>L</i> <i>l</i> VI	573	163	144	152	114	0.94	51.1 ± 2.1	0.5
<i>U</i> <i>u</i> VII	99	21	25	32	21	2.21	57.6 ± 5.0	1.5
<i>H</i> <i>h</i> VII	99	20	26	27	26	0.55	53.5 ± 5.0	0.7
<i>T</i> <i>t</i> VII	99	17	29	19	34	0.01	48.5 ± 5.0	1.7

<sup>a</sup>  $X = Ne$ ;  $x = ne$ ;  $Y =$  the dominant and  $y =$  the recessive of the factor pair shown in Column 1.

<sup>b</sup> The  $\chi^2$ 's of this column, testing independence of  $X$  and  $Y$ , are determined from  $2 \times 2$  tables.

<sup>c</sup> Data showing the independent assortment of  $Ne$  and  $Cf_{N1}$ , the latter of which is situated on Chromosome IV, are given in Tables II and III.

<sup>d</sup> A more accurate treatment of  $ne-h$  data appears on page 54, where the conventional treatment of the hairy stem character as due to a recessive factor has not been followed.

<sup>e</sup> Backcross data for  $Y$ ,  $U$ ,  $H$ , and  $T$ , as well as all the  $F_2$  data, are derived from field populations of 1940, with this exception, that part of the population furnishing backcross data for  $D$ ,  $P$ ,  $O$ , and  $S$ , was grown in the greenhouse.

the remainder, including the laggards of the population, was grown in the field, untrained. Necrosis developed most extensively and characteristically in the greenhouse lot and least extensively in Lot 3. Inasmuch as Lots 1 and 2 each arbitrarily contained half tall and half dwarf plants and thus were not randomly selected, whereas the residual Lot 3 had a great excess of tall plants,

and inasmuch as *ne* proved to be linked with  $d_1$ ,  $p$ ,  $o$ , and  $s$ , it was decided to combine the data from the three lots. Lot 1 could scarcely be considered an unbiased sample of the population. Nevertheless its crossover value for *ne-s*,  $5.9 \pm 0.9\%$ , may well be closer to the true value than is  $8.1 \pm 0.7\%$ . In view of the fairly obvious influence of the environmental conditions in disturbing the ratios it was felt that the most reliable figure for *ne-s* linkage obtainable from the population would be that derived from noting the easily determined *S-s* ratio among plants classified as necrotic. The 490 necrotic plants of this population were classified as 24 with simple inflorescence (*S*) and 466 with compound inflorescence (*s*), yielding a recombination value of  $4.9 \pm 1.0\%$ . Even this figure is probably too high: the incomplete penetrance of *s* (80.6%) suggests that some individuals of genotype *ne/ne; s/s* may appear to be *ne/ne; S/?*, but our experiments do not suggest any explanation of the observed deficiency of *s* plants in this population.

The recombination value of  $45.0 \pm 2.0\%$ , from an  $F_2$  population segregating with respect to the alleles at the *ne* and *h* loci (Table VII), suggests a significant deviation from the figures expected in the absence of linkage. Since, however, the published order of the genes of linkage group VII is *u, h, t* (17), since this order is confirmed by the populations summarized in Table VII, since the figures for the combinations *ne-u* and *ne-t* show no suggestion of linkage, and since *ne* and *h* show a recombination value of 53.5% in the backcross population, there thus remains no suggestion of a linkage between *ne* and *h*, nor of linkage groups I and VII belonging to a single chromosome. An explanation of the apparent lack of independence of *Ne-ne* and *H-h*, as indicated by both  $\chi^2$  and recombination values from  $F_2$  populations, must be sought elsewhere.

A different treatment of the *ne-h* data shows that the lack of independence of the effects of the alleles at or near the *ne* and *h* loci is not as great as the  $F_2$  figures of Table VII indicate. In the preparation of Table VII the conventional treatment of the smooth stem factor as a dominant (17) has been followed. Apparently because of the existence of modifying factors, the expression of the heterozygote, *H/h*, ranges from a moderately hairy condition to a condition in which there are very few long hairs on the plant, but under favorable conditions *H/H*, *H/h*, and *h/h* individuals may be distinguished fairly readily. In the largest of the three  $F_2$  populations providing the 1248 plants used in the calculations reported in Table VII, the *H/H* plants were readily identified but the phenotypes of *H/h* and *h/h* plants were not always distinguishable with certainty. It was felt, therefore, that the classification resulting when the hair condition was considered dominant was more reliable. On the assumption of dominance of the gene for hairiness the *ne-h*  $F_2$  figures of Table VII may be replaced by the following: 569 non-necrotic, hairy; 209 non-necrotic, smooth; 234 necrotic, hairy; 67 necrotic, smooth. The  $\chi^2$  value, calculated from a  $2 \times 2$  table is 2.41 ( $P = 0.12$ ) and the recombination value  $46.5 \pm 2.4\%$ . These data strengthen the conclusion that there is no linkage between *ne* and *h*.

## UNUSUAL DEVIATIONS FROM THEORETICAL SIMPLE MENDELIAN RATIOS

The inheritance of necrosis, as outlined above (p. 41ff), was apparently so clearly established that the author, primarily concerned at the time with linkage studies and with the development of the variety Vetomold, failed to attach any significance to the occurrence of occasional populations in which the proportion of plants developing necrosis was much below expectation. This past spring, when preparing to summarize all data for publication, it was noted that six such populations, each carrying approximately a 3% inheritance from *L. pimpinellifolium*, also carried the *pimpinellifolium* factor *H* (smooth stem), which had been selected through four generations of back-crossing as a prospective marker for a new, Vetomold-like tomato. Inasmuch as these six populations were grown in two widely separated locations in the 1938 field plantation, under conditions giving the best field development of necrosis seen during the course of the investigation, with ratios in numerous lines closely approaching the theoretical values (See Tables III, IV, and the adjacent text), an investigation of other genetically similar populations was indicated.

Table VIII is a collection of the data from all populations, other than  $F_2$  populations from crosses between *L. pimpinellifolium* and *L. esculentum*, resulting from the selfing of individuals carrying the factor *H*, usually in the heterozygous condition, and grown concurrently with other populations in which necrosis developed approximately in accordance with the expected frequency. It may be noted that in all but one small population ( $3P^*$ ) the number of necrotic plants is below expectation, that in six of the 17 families the value of *P* is less than 0.05, that the homogeneity of the 17 populations is great, and that the pooled ratio shows an exceedingly wide deviation from a 3 : 1 ratio.

Table IX similarly assembles data from  $F_2$  populations resulting from crosses between *L. pimpinellifolium* and *L. esculentum*. Although the pooled data for the 17 populations show a reasonable approach to a 3 : 1 ratio the heterogeneity of the populations is very high and the data accordingly suspect. It is obvious that the excess of necrotic plants in the large Tangerine  $F_2$  population nearly balances the highly significant deficiency that appears on the pooling of the remaining 16 populations, as shown at the bottom of the table. The homogeneity of these 16 populations is reasonably good. Thus, with the notable exception of the Tangerine  $F_2$  population, the data indicate that populations derived from the selfing of a hybrid between *L. pimpinellifolium* and *L. esculentum*, as well as those from the selfing of other hybrids of genotype  $Cf_{p_1}/cf_{p_1}$ ;  $Ne/ne$ ;  $H/h$  (Table VIII), show a great deficiency from the number of necrotic individuals expected.

The classification of  $F_2$  populations from crosses between the two species in question has frequently proved difficult, not only with respect to necrosis, but with respect to segregations involving *p*, *f*, *h*, and *wt*, at least. The behavior of such populations indicates the presence of modifying factors. In the case of necrosis the most sharply defined segregations have been



TABLE VIII

THE INHERITANCE OF NECROSIS IN SEGREGATING TOMATO FAMILIES DERIVED FROM THE SELFING OF INDIVIDUALS OF GENOTYPE  $Cf_{P_1}/cf_{P_1}$ ;  $Ne/ne$ ,<sup>a</sup> AND CARRYING THE FACTOR  $H$   
(NOTE: FOOTNOTES <sup>a</sup>, <sup>b</sup>, <sup>c</sup>, OF TABLE II APPLY ALSO TO THIS TABLE)

Year and crop	Family designation	Numbers of immune plants			$\chi^2$ (3 : 1)	$P$ (approx.)
		Total	Non-necrotic	Necrotic		
1938, field	2P <sup>a</sup>	25	21	4	1.080	0.3
1938, field	2P <sup>a</sup>	32	28	4	2.666	0.1
1938, field	3P <sup>a</sup>	15	11	4	0.022	0.9
1938, field	4P <sup>a</sup>	33	27	6	0.818	0.4
1938, field	5P <sup>a</sup>	50	47	3	9.627	<0.01 <sup>c</sup>
1938, field	5P <sup>a</sup>	20	19	1	4.266	0.04 <sup>c</sup>
1938, field	5P <sup>a</sup>	32	29	3	4.167	0.04 <sup>c</sup>
1938, field	5P <sup>a</sup>	33	26	7	0.253	0.6
1938, field	5P <sup>a</sup>	18	17	1	3.630	0.05
1939, spring	7P <sup>a</sup>	21	19	2	2.683	0.1
1939, spring	7P <sup>a</sup>	22	19	3	1.515	0.2
1939, spring	7P <sup>a</sup>	21	19	2	2.683	0.1
1939, spring	7P <sup>a</sup>	18	18	0	6.000	0.01 <sup>c</sup>
1939, fall	7P <sup>a</sup>	27	25	2	4.457	0.04 <sup>c</sup>
1939, fall	7P <sup>a</sup>	27 <sup>a</sup>	24	3	2.777	0.1
1939, fall	8P <sup>a</sup>	26	24	2	4.155	0.04 <sup>c</sup>
1939, fall	8P <sup>a</sup>	27	22	5	0.605	0.4
					51.404	
					42.600	<0.01 <sup>c</sup>
Heterogeneity $\chi^2$ (16 degrees of freedom)					8.804	0.95

<sup>a</sup> In the case of the third last population only, the plant selfed was of genotype  $Cf_{P_1}/Cf_{P_1}$ .

observed in populations resulting after a number of successive backcrosses to esculentum varieties. Here the necrotic individuals are all essentially alike. In  $F_2$  populations of crosses between *L. pimpinellifolium* and *L. esculentum*, however, considerable variation in the degree of development of necrosis has been observed. The 162 plants classified as necrotic at the bottom of Table IX for example, include 27 that did not show striking and typical symptoms of necrosis although they had time to develop such symptoms. Of these, 11 were classified as moderately necrotic, six as slightly necrotic, and one as very slightly necrotic; varying degrees of doubt were expressed regarding the occurrence of necrosis in the remaining nine plants. These 27 plants were rated as necrotic in the present calculation to avoid any suggestion of bias in favor of the hypothesis advanced to explain the results. This variation in the degree of the development of necrosis, when contrasted with the sharply defined segregations in populations whose inheritance is predominantly from *L. esculentum*, indicates the presence, in *L. pimpinellifolium*, of modifying factors, whose effect is the amelioration of the expression of the gene combination  $Cf_{P_1}, ne$ .

TABLE IX

THE INHERITANCE OF NECROSIS IN  $F_2$  TOMATO FAMILIES FROM CROSSES BETWEEN  
*L. pimpinellifolium* AND VARIETIES OF *L. esculentum*

(NOTE: FOOTNOTE ° OF TABLE II APPLIES TO THIS TABLE)

Year and crop	Esculentum parent	Numbers of immune plants			$\chi^2$ (3 : 1)	P (approx.)
		Total	Non-necrotic	Necrotic		
1938, field	Acme	46	31	15	1.420	0.2
1938, field	Banalbufar	43	33	10	0.070	0.8
1938, field	Beefheart	34	27	7	0.353	0.5
1938, field	Bonny Best	50	43	7	3.227	0.05
1939, field	Break O'Day	76	61	15	1.123	0.3
1938, field	Crackerjack	44	31	13	0.485	0.5
1938, field	Earliana	42	36	6	2.571	0.1
1938, field	Early Detroit Purple	44	34	10	0.121	0.7
1938, field	First of All	46	38	8	1.420	0.2
1938, field	Grand Rapids	88	75	13	4.909	0.03°
1938, field	Imperial Globe	44	39	5	4.364	0.04°
1938, field	Potentate	85	78	7	12.742	<0.01°
1939, field	Rouge Naine Hative	93	76	17	2.240	0.1
1938, field	Sandblast	44	32	12	0.121	0.7
1939, field	Tangerine	1079	778	301	4.827	0.04°
1938, field	Tuckswood Favourite	44	34	10	0.121	0.7
1938, field	Wonder of Italy	44	37	7	1.940	0.2
					42.054	
					1.514	0.2
Heterogeneity $\chi^2$ (16 degrees of freedom)					40.540	<0.01°
Totals, less Tangerine $F_2$		867	705	162	37.277	<0.01°
					18.440	
Heterogeneity $\chi^2$ (15 degrees of freedom)					18.787	0.2

Table X assembles data from 19 backcross populations carrying the factor  $H$  and drawn from eight different crops. A comparison of Table X with Table II, which assembles all backcross data, including those given in Table X, shows clearly that in backcross populations there is no evidence of a deficiency of necrotic individuals in the presence of the factor  $H$ .

The available data dealing with the relationship between  $ne$  and Chromosome VII are admittedly inadequate but they are offered now because of the lack, in the present circumstances, of an opportunity to carry out the necessary progeny tests. The factor  $ne$  has been located on Chromosome I, while  $H$  is located on Chromosome VII, between  $U$  and  $T$  (17). These circumstances, together with the data of Tables VIII, IX, and X, indicate that there is a failure of the development of necrosis in some immune plants of genotype  $ne/ne$ , in selfed populations, and that this is due to the factor  $H$  and (or) one or more genes near it. The fact that many plants known to be of genotype  $H/H$  develop necrosis suggests that a gene or genes near  $H$  on Chromosome VII constitute an essential part of the inheritance that modifies the expression of

TABLE X.

THE INHERITANCE OF NECROSIS IN SEGREGATING TOMATO FAMILIES DERIVED FROM BACKCROSSING HYBRID IMMUNE, NON-NECROTIC, SMOOTH-STEMMED INDIVIDUALS TO ESCULENTUM VARIETIES, I.E. CROSSES OF THE TYPE  $Cf_{p_1}/cf_{p_1}; Ne/ne; H/h \times cf_{p_1}/cf_{p_1}; ne/ne; h/h$   
(NOTE: FOOTNOTES <sup>a</sup>, <sup>b</sup>, <sup>c</sup> OF TABLE II ALSO APPLY TO THIS TABLE)

Year and crop	Family designation	Number of immune plants			$\chi^2$ (3:1)	P (approx.)
		Total	Non-necrotic	Necrotic		
1936, spring	2P	25	13	12	0.040	0.8
1938, field	2P	5	3	2	0.200	0.6
1938, field	2P	11	9	2	4.456	0.04 <sup>c</sup>
1939, field	2T <sup>d</sup>	99	46	53	0.495	0.5
1939, field	2T	29	14	15	0.034	0.85
1938, field	3P	13	6	7	0.077	0.8
1937, spring	4P	4	3	1	1.000	0.3
1938, field	4P	17	5	12	2.883	0.1
1937, fall	5P	18	7	11	0.889	0.3
1938, field	5P	18	7	11	0.889	0.3
1938, spring	6P	7	5	2	1.286	0.3
1938, spring	6P	9	6	3	1.000	0.3
1938, field	6P	15	5	10	1.667	0.2
1938, field	6P	15	8	7	0.067	0.8
1938, field	6P	13	9	4	1.923	0.2
1938, fall	7P	18	8	10	0.222	0.6
1938, fall	7P	18	8	10	0.222	0.6
1939, spring	8P	10	4	6	0.400	0.5
1939, fall	8P	18	14	4	5.555	0.02 <sup>c</sup>
					23.305	
					0.011	0.9
Heterogeneity $\chi^2$ (18 degrees of freedom)					23.294	0.2

<sup>d</sup> The Symbol T refers to the variety Tangerine.

the factor combination  $Cf_{p_1}, ne$  in a chromosome complex derived predominantly from *L. esculentum*. There has been no suggestion that the factor *H* itself has such an effect.

### Discussion

Physiologically, the present studies have not gone beyond the demonstration that light intensity and temperature have a marked effect upon the extent to which autogenous necrosis of tomatoes develops. The leaf symptoms of necrosis, its non-parasitic nature, and the clearly established reduction of its severity through the shading of potentially necrotic plants suggest that the careful study of such physiological characteristics as the transpiration rates of immune non-necrotic and immune necrotic segregants, among the otherwise uniform members of populations resulting from repeated backcrosses to one variety might yield valuable results.

Throughout this article the symbols  $Cf_{p_1}$ ,  $cf_{p_1}$ , *Ne*, and *ne* have been considered to refer to single genes. It is realized, however, that the difference

between immune non-necrotic individuals and immune necrotic individuals may not be due simply to the difference between one pair of alleles. It is quite conceivable, for instance, that the author's *ne* might actually be a very short deficiency in Chromosome I or that his *Ne* might be a very small, localized gene complex. The frequent confusion of gene mutations and deficiencies is discussed in some detail by Goldschmidt (9) in a chapter on the nature of the gene, and in any case is well known to geneticists. Mangelsdorf (18, pp. 161-207), investigating the differences between teosinte and maize through repeated backcrosses to maize, following an original cross between maize and teosinte, has demonstrated segments of chromatin that differentiate teosinte from maize, these segments behaving almost as though they were simple Mendelian factors. In one case, for instance, a backcross resulted in the production of 64 plants with the effect of a certain teosinte segment and 66 plants without these effects. This segment showed linkage with the factor *A* on Chromosome III, and a recombination value of 25%. Other segments broke into smaller fragments, to a certain extent. It is apparent that segment size and frequency of opportunities for crossing over would determine the extent to which the behavior of a chromosome segment might simulate the behavior of a gene. There has been no evidence, in this investigation of necrosis, that we are not dealing with the interaction of the members of two pairs of alleles but these other possibilities are mentioned as a qualification of the author's statement of the simplicity of the inheritance of the normally encountered difference between necrotic and non-necrotic plants immune from *C. fulvum*.

The genetic investigation of necrosis has yielded results of both practical and theoretical importance. In 1942 the author (16) stated that "The Vetomold tomato is essentially the esculentum variety Potentate except for two dominant genes derived from *Lycopersicon pimpinellifolium*: *Cf<sub>p1</sub>*, conferring immunity from *Cladosporium fulvum* strains 1 to 4; and *Ne*, conditioning the compatibility of *Cf<sub>p1</sub>* with esculentum chromosome complexes". The contrasting incompatibility, of which the development of symptoms of necrosis is the visible expression, has been found in the combination of the genotype *Cf<sub>p1</sub>/?*; *ne/ne* with a chromosome complex derived largely from *L. esculentum*, whereas the combination of the genotype *Cf<sub>p1</sub>/?*; *Ne/?* with an esculentum chromosome complex results in the production of non-necrotic individuals. The incompatibility appears to be between *Cf<sub>p1</sub>* and an esculentum chromosome complex and not between extensive portions of the chromosome complexes of *L. pimpinellifolium* and *L. esculentum*. It is well known that these two species hybridize readily, producing highly fertile hybrids and there is no suggestion of the type of incompatibility, encountered in many interspecific plant hybrids, which results in partial or complete sterility. No plant of genotype *cf<sub>p1</sub>/cf<sub>p1</sub>* has developed necrosis. Possibly the combination of the genotype *Cf<sub>p1</sub>/?*; *ne/ne* with a pimpinellifolium chromosome complex would also cause the production of necrosis, but time and space have not been available for the development of this genotype.

The cause of the reduction in the proportions of necrotic plants in populations resulting from the selfing of heterozygous immune, heterozygous non-necrotic plants, bearing the factor *H* (smooth stem) is not clear. The most striking feature of these tests involving *H* is that backcrosses to *L. esculentum* yielded results in accordance with the expectation of 1 immune non-necrotic: 1 immune necrotic while populations resulting from selfing gave significantly less than  $\frac{1}{4}$  necrotic individuals among the immune segregants. Among those populations whose inheritance was derived predominantly from *L. esculentum* the populations from selfing would contain individuals homozygous with respect to *Cf<sub>p1</sub>*, *H*, and a few chromosome segments derived from *L. pimpinellifolium* and located adjacent to the loci of *Cf<sub>p1</sub>* and *H* on Chromosomes IV and VII, respectively, while individuals from backcrosses would lack such homozygosity: otherwise the two types of populations are essentially alike. It is thus indicated that some gene or genes in the homozygous condition (and presumably located in the vicinity of the *H* locus) may prevent the development of necrosis in plants having the gene combination *Cf<sub>p1</sub>*, *ne*. An alternative possibility is that such a gene or genes in the heterozygous condition may prevent the development of necrosis when *Cf<sub>p1</sub>* is in the homozygous condition. Further experiments are required to elucidate the nature of the observed effect.

It is interesting to speculate concerning the evolution of the immunity of *L. pimpinellifolium* from *C. fulvum* and of its freedom from necrosis. A hypothesis that appeals to the author is that an ancestor of our strain of *L. pimpinellifolium*, susceptible to *C. fulvum*, evolved an immunity from certain prevalent races of this fungus, *Cf<sub>p1</sub>* being the key factor conferring this immunity. A second result of the presence of this factor was the development of a certain amount of necrosis, its severity depending on the fluctuating environmental conditions. Various factors, from new or from earlier mutations, which ameliorate the severity of the necrosis, were incorporated into the strain through natural selection and such factors are responsible for the variable development of necrosis, from plant to plant, in an *F<sub>2</sub>* population from a cross between our strain of *L. pimpinellifolium* and various varieties of *L. esculentum*. Among factors modifying the expression of the factor combination *Cf<sub>p1</sub>*, *ne*, one or a group of a few closely linked factors on Chromosome VII near the *H* locus resulted in the complete suppression of necrosis in some immune plants; the evolution of the single factor, *Ne*, likewise provided for the complete inhibition of necrosis. The factor *Ne* and its recessive allele *ne* govern the simple Mendelian inheritance of necrosis.

There is a temptation to reason that modifiers of the combination *Cf<sub>p1</sub>*, *ne* would evolve after the establishment of this gene combination, and certainly it may be reasoned that modifiers that improve the fitness of a plant and thus have survival value are more likely to be retained after than before the development of the factor or group of factors that they modify. In this connection Harland's (13) experiments with cotton are interesting and instructive. Working with crosses between a 'crinkled dwarf' strain of Sea Island cotton

(*Gossypium barbadense*) and various strains of *G. hirsutum*, Harland was able to demonstrate, in certain strains of *G. hirsutum*, groups of modifying factors, distinct from similar factors in Sea Island cotton, whose effect was to make the crinkled dwarf mutant recessive. As this mutant has occurred repeatedly in Sea Island cotton but has never been observed in *G. hirsutum*, Harland's data show very clearly that groups of modifying factors may be incorporated in the inheritance of a plant quite independently of the factor or factors that they modify. Harland concludes that modifiers of dominance are of advantage to the wild type and are thus selected on their own account. His data thus indicate that it cannot be assumed that the modifying factors of *L. pimpinellifolium* here discussed were incorporated in immune strains of this species after the development of the factor  $Cf_{p_1}$ . *Ne*, too, may well have originated before  $Cf_{p_1}$ .

Considerable experimentation would be necessary to test the validity of certain points in the hypothesis given. For instance, we have no knowledge of the expression of the gene combination  $Cf_{p_1}$ , *ne* in the tropical lowlands of Peru, one of the natural habitats of *L. pimpinellifolium* (19), but our experience suggests that severe necrosis might develop in this habitat. The investigation of the modification of the expression of the factor combination  $Cf_{p_1}$ , *ne* by the presumed factor or factors near *H* on Chromosome VII is in its preliminary stages only but could be extended readily. The study of plants with the combination  $Cf_{p_1}$ , *ne* in a *pimpinellifolium* chromosome complex might well throw light on this interesting evolutionary problem but such plants have yet to be developed. All our populations trace from a single collection of *L. pimpinellifolium*. The study of the breeding behavior of additional collections of this and other species of *Lycopersicon* that cross with *L. esculentum* may well be a fruitful one and it will be interesting to see whether either of the gene combinations  $Cf_{p_1}$ , *ne* or  $Cf_{p_1}$ , *Ne* is discovered, particularly in *L. pimpinellifolium*. Alexander, Lincoln, and Wright's (3) survey of the genus *Lycopersicon* for resistance to organisms parasitic on *L. esculentum* is a step in this direction. Others who are breeding tomatoes for resistance to *C. fulvum* may possibly soon provide us with additional data pertinent to these problems. It is hoped that the hypothesis offered may direct the attention of other workers to some of these problems.

Reference has been made to the brief reports of Guba (12) of a "hereditary breakdown" and of Alexander (1) of "what appeared to be a factor for a partial lethal" among  $F_2$  and later derivatives of crosses between *L. pimpinellifolium* and *L. esculentum*. These conditions are almost certainly identical with our 'necrosis' but our data show that the association of necrosis and immunity is not due to linkage, as Alexander reported, but to a factor interaction. The demonstration that the development of necrosis, a serious abnormality superficially resembling somewhat a disease caused by a parasite, may be controlled by members of two factor pairs only, seems to be without a close parallel in other organisms. The author has not been able to find any reference to an abnormality of this type that has been related clearly to the

effect of precise genes. The closest parallel known to him is that of the inheritance of melanism in the platyfish, *Platypoecilus maculatus*, and the swordtail, *Xiphophorus hellerii*, which has been investigated extensively by Gordon (10) and Gordon and Smith (11). Spotted races of *P. maculatus* owe their spotting to the presence of many macromelanophores (large black pigment cells) in its skin, this condition being controlled by a dominant, sex-linked gene, *Sp*. In crosses between spotted and unspotted varieties of *P. maculatus* no unusual results have been encountered. However, a cross of a spotted *P. maculatus* (*Sp/Sp*) with an unspotted variety of the swordtail, *X. hellerii* (*sp/sp*) yields  $F_1$  hybrids (*Sp/sp*) that show a much greater extension of the black pigment than in *P. maculatus* of genotypes *Sp/sp* or *Sp/Sp*. Such hybrids, characterized as melanotic, have tumor-like growths due to the greatly accelerated growth of the macromelanophores. When these hybrids are backcrossed to *P. maculatus* (*Sp/Sp* or *Sp/sp*) the hybrids carrying the factor *Sp* are but slightly melanotic; when they are backcrossed to *X. hellerii* (*sp/sp*) the *Sp/sp* hybrids develop intensified melanosis. Gordon's data show quite clearly that the unfavorable effects of the gene *Sp* are due to its association with an undetermined number of genes from *Xiphophorus*. Somewhat similarly, the abnormality here designated as necrosis is caused by the pimpinellifolium factor *Cf<sub>p1</sub>*, in association with an undetermined number of genes from *L. esculentum*, including *ne* in the homozygous condition.

The autogenous nature of at least some of the cases of necrosis of the leaves of the Chiefkan and Reliant varieties of wheat and of several hybrid lines of wheat has been reported by Chester (7), although there has been no elucidation of the inheritance of such necrosis. Necrotic leaf spotting, commonly known as 'physiological leaf spot' has been observed in certain varieties of wheat, oats, and barley. Chester found that the leaves of plants of most of 80 hybrid lines of wheat developed small watersoaked spots upon being held overnight at 100% humidity in an inoculation chamber but that in most lines these spots disappeared soon after the chamber was aerated in the morning. In other lines the spots remained and became necrotic. In the field only the replicas of these latter lines, and such varieties as Chiefkan, developed 'physiological leaf spot'. Chester concludes that "some wheat strains lack the physiological ability to recover, without injury, from watersoaking due to high humidity, and that this inherited fault is the cause, at least in some cases, of 'physiological leaf spot'". Chester's data, in conjunction with our own data on environmentally induced variations in the expression of necrosis, suggest the possible fruitfulness of a study of the water relations of potentially necrotic tomato plants. It is of interest also to note that the 80 lines of wheat hybrids investigated by Chester had been developed for resistance to *Puccinia triticina*, and to recall that necrosis in tomatoes occurs only in the presence of an immunity factor *Cf<sub>p1</sub>*. It is quite possible that the spotting in Chester's lines of wheat is one expression of a factor or factors conferring disease resistance.

From 1932 (20) until 1939 the problem of breeding tomatoes for resistance to *Cladosporium fulvum* seemed to be a relatively simple one, except while a method of eliminating necrosis was sought for. The discoveries, from 1939 onwards, of 'new' strains of *C. fulvum* capable of infecting and developing readily upon Vetomold and other similar introductions of the genotypes  $Cf_{p_1}/Cf_{p_1}; Ne/Ne$  (2, 5, 12, 4) has complicated this problem greatly and initiated a search for additional factors conferring resistance to the 'new' strains of *C. fulvum*. Unless there are discovered other factors that confer immunity from *C. fulvum* races 1 to 4, but do not contribute to the development of necrosis, necrosis will continue to be important in breeding programs because of the widespread distribution of races 1 and 3 of the pathogen. Whenever Vetomold or other similar, immune, non-necrotic varieties are crossed with pure esculentum varieties necrosis may be expected among the immune segregants of the second generation. Clarke and Sherrard (8) have already reported such occurrences following crosses with Vetomold. Their 'leaf spot' is undoubtedly our 'necrosis'. The enthusiasm with which Vetomold is regarded, horticulturally, in some localities and its poor performance in others accords with the opinion of many workers that tomato breeding is a somewhat regional problem. The dominance of the two factors conditioning the development of immunity from *C. fulvum* physiologic races 1 to 4 and of non-necrotic foliage makes the backcross method of developing new varieties to suit local conditions a convenient one.

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# FURTHER STUDIES ON THE EFFECT OF LEAF RUST ON THE YIELD, GRADE, AND QUALITY OF WHEAT<sup>1</sup>

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## Abstract

Experiments were carried out at Winnipeg in 1944, 1945, and 1946 to ascertain the effects of leaf rust (*Puccinia triticina* Erikss.) on the yield, grade, and quality of wheat. Infection in the rusted plots ranged from 22% on some varieties to 87% on others. In these tests, reductions of as much as 26% in yield of straw, 40% in yield of seed, 27% in kernel weight, and 3.5 lb. in bushel weight were caused by infections ranging from 78% to 87%. Lighter infection caused smaller but important reductions. Heavy infection generally reduced grades by one commercial grade while light to moderate infection caused no grade reduction. With but one exception, leaf rust decreased the percentage protein content of the seed, although in the majority of cases it increased baking strength as measured by loaf volume. The carotinoid content of the seed was invariably increased by leaf-rust infection.

## Introduction

Leaf rust of wheat (*Puccinia triticina* Erikss.) occurs in Western Canada every year. In some years it spreads through much of the grain growing area of the three Prairie Provinces, and frequently becomes very prevalent in Manitoba and eastern Saskatchewan, where, as has been shown in previous experiments carried out by the authors (5, 6), it not uncommonly causes appreciable crop losses. Since none of the bread wheat varieties presently grown in the rust area of Western Canada possesses much resistance to leaf rust, and the prospects for the early introduction of a highly leaf-rust resistant variety are not very promising, it was decided to continue the investigations initiated in 1938 to determine the effect of leaf rust on the yield and quality of wheat. The results of the experiments carried out in 1944, 1945, and 1946 are given in this paper.

## Methods

The experiments were conducted in the field in adequately replicated rod-row plots. One-half of the plots of each variety, in each test, were kept as free from rust as possible by frequent applications of sulphur dust during the growing season. The dust was applied to the plots three times a week at the rate of 30 lb. per acre per dusting from the time rust began to appear in the plots until shortly before the plants matured. The non-dusted plots, in the test of 1944, were artificially inoculated with leaf rust when the plants had reached the fifth-leaf stage. However, in the tests of 1945 and 1946 rust

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inoculum was not artificially introduced into the plots and they became infected by naturally occurring inoculum. The yield of seed, the weight per bushel, the weight per 1000 kernels, and the percentage of protein were determined for each individual plot in each test. In addition, the yield of straw was determined for each plot in the test of 1944. Composite samples of the grain harvested from each lot of plots were graded and tested for milling and baking quality.

The samples were graded by grain inspectors of the Western Grain Inspection Office, Winnipeg, Man. The milling and baking tests were carried out in the Milling and Baking Laboratory of the Cereal Division, Experimental Farms Service, Ottawa. The protein determinations were made in the Chemical Laboratories, Science Service, Ottawa.

## Results

### *Severity of Infection in the Plots*

In 1944, leaf-rust infection in the plots became established early and the highly susceptible varieties Saunders and Thatcher were very heavily rusted. The somewhat less susceptible variety, Regent, carried comparatively light infection. In the 1945 test, infections again appeared early and all the varieties tested became heavily rusted. Weather conditions in 1946 were unfavorable for rust development and infections were later in becoming established and were less severe than in the two previous years. Rust was effectively controlled in the dusted plots in all three years and no more than trace infections developed.

### *Effect on Yield, Kernel Weight, and Bushel Weight*

Leaf rust adversely affected the yield of straw of all the varieties in the tests of 1944, the only test for which straw yields were taken, and reduced the yield of seed, kernel weight, and bushel weight of all the varieties in the tests in 1944, 1945, and 1946. In 1944 and 1945, these adverse effects were very pronounced and the reductions were in all cases statistically significant. But in 1946, when rust infections were later in getting established and lighter than in the two previous years the reductions in yield, kernel weight, and bushel weight were small and, with the exception of those for kernel weight, nonsignificant. These findings are substantially in accord with the results of earlier experiments made by the present authors (5, 6) and are in agreement with those of Johnston and Miller (3), Mains (4), and Waldron (7) all of whom found that heavy leaf-rust infection adversely affected yield, kernel weight, and bushel weight. The yield and other relevant data for the experiments made in 1944, 1945, and 1946 are given in Table I.

### *Effect on Protein Content of the Seed*

The percentage of seed protein of the varieties was significantly reduced by leaf rust in both the 1944 and 1945 experiments. In the test of 1946 it reduced the seed protein of Thatcher and Marquis slightly but had no appreciable effect on the seed protein of Redman. Of 36 paired samples from

TABLE I

THE EFFECT OF LEAF RUST ON THE YIELD OF STRAW, YIELD OF SEED, 1000-KERNEL WEIGHT, BUSHEL WEIGHT, AND GRADE OF WHEAT VARIETIES GROWN IN FIELD PLOTS AT WINNIPEG IN 1944, 1945, AND 1946

Variety	Treat- ment	Amount of infection	Average yield of straw per plot, oz.	Reduc- tion in yield of straw, %	Average yield of seed per acre, bu.	Reduc- tion in yield of seed, %	Average weight per 1000 kernels, gm.	Reduc- tion in kernel weight, %	Average weight per bu., lb.	Grade (Northern)
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*Experiment of 1944*

Saunders	Dusted	Trace	63.9		52.3		32.3		61.2	2
Saunders	Rusted	78.0	49.1	23.2	32.3	38.2	23.4	27.5	57.5	3
Thatcher	Dusted	Trace	72.5		53.6		30.5		60.0	2
Thatcher	Rusted	87.0	53.5	26.2	31.8	40.7	22.4	26.5	58.5	3
Regent	Dusted	Trace	67.8		50.0		35.5		63.1	2
Regent	Rusted	22.0	60.3	11.1	41.7	16.6	30.1	15.2	61.7	2
Necessary difference <sup>a</sup>			4.9		4.5		1.8		0.9	

*Experiment of 1945*

Thatcher	Dusted	Trace	—	—	58.5		27.8		61.4	3
Thatcher	Rusted	82.0	—	—	41.0	29.3	20.4	22.6	59.8	3
Thatcher Selection	Dusted	Trace	—	—	66.4		28.8		62.8	3
Thatcher Selection	Rusted	77.0	—	—	44.9	31.8	21.4	25.7	60.0	3
Hope	Dusted	Trace	—	—	38.9		32.4		59.4	3
Hope	Rusted	52.0	—	—	27.2	30.8	24.6	28.1	57.0	4
Necessary difference <sup>a</sup>					11.3		2.4		1.5	

*Experiment of 1946*

Thatcher	Dusted	Trace	—	—	66.5		30.2		63.1	1
Thatcher	Rusted	65.0	—	—	61.1	8.8	26.2	13.3	62.4	2
Redman	Dusted	Trace	—	—	60.6		36.4		62.1	1
Redman	Rusted	45.0	—	—	60.1	0.8	35.2	3.3	62.1	2
Marquis	Dusted	Trace	—	—	55.0		35.2		63.4	1
Marquis	Rusted	55.0	—	—	46.2	16.0	31.4	10.8	63.4	2
Necessary difference <sup>a</sup>					<sup>b</sup>		0.45		<sup>b</sup>	

<sup>a</sup> Necessary difference, 5% level of significance, between means of dusted and rusted plots within a given variety.

<sup>b</sup> Differences between dusted and rusted plots nonsignificant.

the rusted and nonrusted plots in the 1944 and 1945 tests, the percentage of seed protein in all but one instance was lower for the rusted sample than for the corresponding nonrusted one. Similar results were obtained by the authors (5) in experiments made in 1938, as well as by Caldwell *et al.* (2) in 1930, and by Waldron (7) in 1935 who found that leaf rust reduced seed protein. However, in experiments made by the authors (6) in 1940, samples from plots that had been affected by leaf rust were higher in protein content than corresponding nonrusted samples. Similar results were obtained by Broadfoot (1) in 1927. The data on protein analysis are given in Table II.

### *Effect on Milling and Baking*

Although leaf rust decreased kernel weight and bushel weight it did not cause a parallel reduction in flour yield in so far as experimental milling tests could reveal. Since only one sample was milled from each treatment the experimental error was too large to show small differences.

Leaf rust definitely increased the carotinoid content of the flour and decreased the score for crumb color in the 1944, 1945, and 1946 experiments.

Despite the lower seed protein of the rusted samples the loaf volumes were not lower and in some instances were higher for these samples than for the corresponding non-rusted samples. In all three years mixogram curves on flour-water doughs made from the rusted sample of each variety showed stronger characteristics than those from the corresponding nonrusted sample. Since the percentage of wheat protein was lower for the rusted sample it would be expected that the loaf volume would be lower. But since this was not usually the case, it might be concluded that the quality of the gluten in the flour from the rusted samples was superior. The mixogram curves lend support to that assumption. In experiments made with the 1938, 1940, and 1943 crops it was found that leaf-rust infection did not usually reduce loaf volume; in the majority of instances it improved it. The milling and baking data are presented in Table II.

### *Effect on Grade*

Leaf rust affects grades chiefly by lowering bushel weight. As light leaf-rust infections seldom affect bushel weight appreciably they usually do not have any effect on grade. Heavy leaf-rust infections, on the other hand, particularly when established early, often reduce bushel weights by several pounds and naturally affect grades adversely. Grades, however, are affected by many factors and some of these may obscure or nullify the effects of leaf rust. This happened in the 1945 test when the nonrusted samples, although exceeding the corresponding rusted ones from 1.6 to 2.8 lb. per bushel, were, owing to the presence of green kernels, placed in the same grade as the lightly rusted samples. In the 1944 test the rusted samples of the susceptible varieties were reduced by 3.5 lb. in bushel weight and were reduced in grade by one commercial grade. In 1946 the rusted samples of the three varieties, Thatcher, Redman, and Marquis, graded one commercial grade lower than corresponding nonrusted samples. However, as the rusted samples were

TABLE II

THE EFFECT OF LEAF RUST ON THE FLOUR YIELD, FLOUR COLOR, PERCENTAGE OF SEED PROTEIN, LOAF VOLUME, CRUMB TEXTURE, AND CRUMB COLOR OF WHEAT VARIETIES GROWN IN FIELD PLOTS AT WINNIPEG IN 1944, 1945, and 1946

Variety	Treat- ment	Rust infection, %	Flour yield, %	Flour color (carotene), p.p.m.	Seed protein, %	Loaf volume, cc.	Crumb texture	Crumb color
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*Experiment of 1944*

Saunders	Dusted	Trace	76.7	1.6	14.7	913	7.5	—
Saunders	Rusted	78.0	72.6	2.3	13.9	888	7.5	—
Thatcher	Dusted	Trace	72.7	1.3	15.0	870	8.3	—
Thatcher	Rusted	87.0	71.7	2.1	13.5	813	7.5	—
Regent	Dusted	Trace	72.5	1.2	15.8	913	8.5	—
Regent	Rusted	22.0	72.7	1.5	14.7	993	6.5	—
Necessary difference <sup>a</sup>					0.33			

*Experiment of 1945*

Thatcher	Dusted	Trace	75.8	1.7	15.5	795	7.5	7.0
Thatcher	Rusted	82.0	75.2	2.3	14.7	885	7.5	6.3
Thatcher Selection	Dusted	Trace	74.8	1.6	15.2	840	8.0	8.5
Thatcher Selection	Rusted	77.0	75.6	2.2	14.3	870	8.0	8.3
Hope	Dusted	Trace	76.0	1.7	16.2	795	8.0	8.5
Hope	Rusted	52.0	75.2	2.3	15.5	830	8.0	8.0
Necessary difference <sup>a</sup>					0.49			

*Experiment of 1946*

Thatcher	Dusted	Trace	70.8	1.6	15.1	835	7.8	7.0
Thatcher	Rusted	65.0	72.6	2.0	14.7	885	7.8	6.3
Redman	Dusted	Trace	74.8	1.3	15.2	875	8.0	8.5
Redman	Rusted	45.0	74.6	1.4	15.5	940	8.0	8.3
Marquis	Dusted	Trace	71.9	1.3	16.1	945	8.0	8.5
Marquis	Rusted	55.0	74.1	1.5	15.4	915	8.0	8.0
Necessary difference <sup>a</sup>					b			

<sup>a</sup> Necessary difference, 5% level of significance, between means of dusted and rusted plots within a given variety.

<sup>b</sup> Difference between dusted and rusted plots nonsignificant.

degraded on account of the presence of immature kernels in the sample but not because of reduction in bushel weight or for any other condition attributable to the effect of leaf rust, it must be assumed that the grade reductions of the rusted samples, in that year, were brought about by causes other than leaf rust.

### Discussion

These experiments indicate that light leaf-rust infections have little or no effect on grade, but that heavy infections, when other grade influencing factors do not interfere, may cause grade reductions. Leaf rust apparently has a marked effect on the carotinoid content of the grain, for the rusted samples in every test were appreciably higher in yellow pigment than the nonrusted ones. Although the rusted samples were usually, but not always, lower in percentage of protein than the nonrusted samples, they possessed, in the majority of cases, greater baking strength and produced loaves of greater volume than did the nonrusted samples. The rust apparently, in as yet some unexplained manner, favorably modified protein quality.

The effects of leaf rust on kernel weight, bushel weight, and yield were more pronounced than those on quality and grade. Heavy infections invariably caused very substantial kernel weight, bushel weight, and yield reductions, and even a moderately light infection (22%) such as occurred on Regent in the test of 1944, caused appreciable reductions. This demonstrated ability of leaf rust to injure the crop even when present in only moderate amounts suggests that the economic importance of this rust may be underestimated. Small but significant reductions such as those suffered by Regent in the above-mentioned test would certainly remain undetected under ordinary farm practices.

As no approved commercial bread wheat varieties possessing high leaf-rust resistance are now extant it would appear most desirable to make such varieties available to growers at the earliest time possible. The work now in progress with that end in view, therefore, should now receive added emphasis.

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# WINTER CROWN ROT OR SNOW MOLD OF ALFALFA, CLOVERS, AND GRASSES IN ALBERTA

## I. OCCURRENCE, PARASITISM, AND SPREAD OF THE PATHOGEN<sup>1</sup>

BY M. W. CORMACK<sup>2</sup>

### Abstract

Winter crown rot or snow mold of alfalfa, clovers, and grasses, caused by an unidentified low-temperature basidiomycete, is widespread and often of major importance in the central and northern areas of Alberta and Saskatchewan. For example, during a six year period an average of 62% of the alfalfa stands examined in west-central Alberta were affected, and the average estimated damage was 10%. Severe damage also occurs in alsike and white Dutch clover, and in timothy, red top, and creeping red fescue. The disease is less prevalent in red clover, Kentucky bluegrass, and meadow fescue, and seldom occurs in brome grass, crested wheat grass, and slender wheat grass. Iris and other garden perennials, as well as dandelion, quack grass, and various wild plants are also damaged.

The pathogen attacks the dormant plants beneath the snow during the first thaw in the late winter or early spring. The plants are killed or weakened in irregular patches as the result of rotting of the crown buds and tissues. These symptoms are distinct from those of true winter killing, with which the damage has been confused. The pathogen is difficult to isolate, except at a temperature near freezing from superficial mycelium or freshly infected tissues.

The results of infection experiments in the field and under controlled conditions indicate that the development of the disease is associated with physiological changes in the host. Inoculated alfalfa plants brought inside at weekly intervals became susceptible at dates varying from late November to late December in different seasons. Infection was most severe under the conditions provided by a slowly melting snow cover and was also influenced by soil temperature, soil moisture, and growth of the pathogen.

The pathogen appears to spread mainly by means of mycelium, since no sporulating stage has been found. The mycelium spreads both above and below ground at the time of the first spring thaw. The distance of radial spread, as measured by killing of the plants, varied in different years from 2.7 to 7.5 in. in dense alfalfa stands, and from 0.5 to 6.0 in. in bare land.

Winter crown rot is the name now proposed for a previously undescribed disease of alfalfa and other forage crops that is prevalent and sometimes very destructive in Alberta and Saskatchewan. It is commonly called snow mold in turf grasses (1). This disease, frequently confused with true winter killing, is caused by an unidentified low-temperature basidiomycetous fungus (2) that attacks the dormant plants beneath the snow when thawing first starts in the late winter or early spring. As in the case of *Plenodomus Meliloti*, studied by Sanford (7), the fungus ceases to cause damage before growth is resumed. The pathogen is more virulent and injurious to alfalfa and clovers than any of the root- and crown-rotting fungi previously studied in Alberta (3).

Considerable information has been obtained on winter crown rot during the past seven years, but several phases of the investigation are not yet completed.

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This first paper of a series will deal primarily with the disease symptoms, distribution, parasitism, and manner of spread of the pathogen. The results of field studies on host range, varietal reaction, persistence, and control, and on the cultural characteristics and taxonomy of the pathogen, will be reported later.

### Symptoms

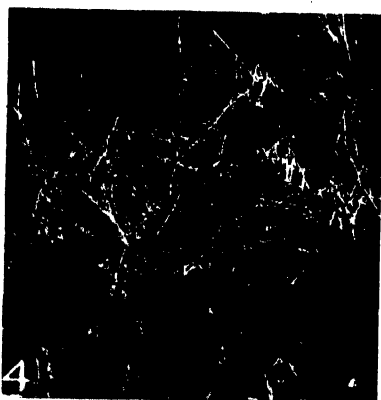
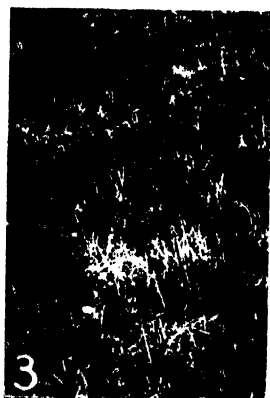
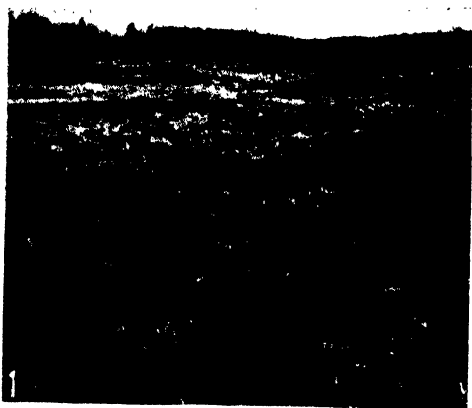
The occurrence of dead plants in irregular patches (Figs. 1, 2, 10, 11) is the most characteristic symptom of winter crown rot damage in alfalfa and clover stands in the central and northern areas. These dead patches first become evident in the spring when growth starts in adjacent unaffected areas. They are most common on slopes, but may occur at random in a level field. Scattered individual plants or small patches (Figs. 3, 4) are usually killed first, forming a starting point for larger patches that extend from year to year. Large patches may be killed in a single year if winter or early spring conditions are particularly favorable for the disease. Dandelions or other weeds soon occupy the bare areas (Fig. 5). In southern Alberta the winter crown rot pathogen most commonly causes partial rotting of the crowns and weakening of alfalfa plants, rather than killing in patches as in the central and northern areas. This damage is often not very evident unless the plants are examined closely or dug up.

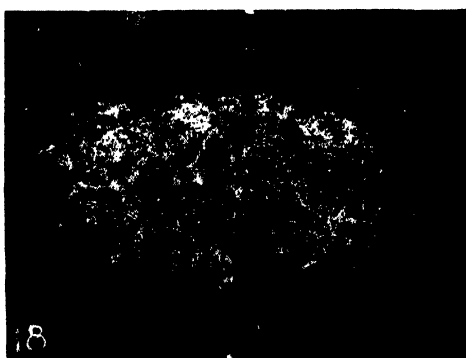
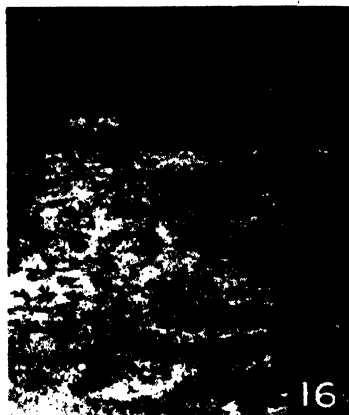
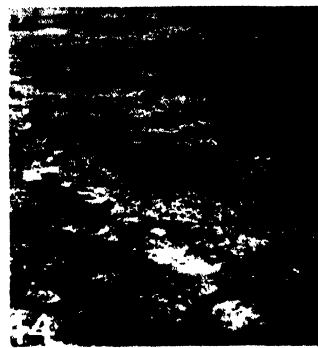
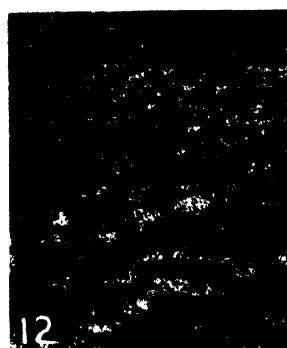
An alfalfa or clover plant affected by winter crown rot has dark brown rotted areas at the crown, and, less commonly, lower on the root (Figs. 6 to 9). This damage is apparently distinct from that reported by Jones (5) in alfalfa clones in Wisconsin. The extent of the development of lesions varies greatly in different seasons and localities. Plants are often killed or greatly weakened when only the crown buds and a very small portion of the underlying tissues are affected (Fig. 6). At the other extreme, the entire crown and upper portion of the root may be severely rotted (Fig. 7). Even when the plant is killed, the main root usually remains relatively sound until natural decay sets in. When the crown is not completely rotted, some of the buds usually escape and start weak growth late in the spring (Fig. 6). This may result in proliferation of a portion of the crown during the growing season (Fig. 8). Another sign of previous infection is the coarse, whitish mycelial remnants of the pathogen sometimes found on or near the crowns of dead or damaged plants.

Winter crown rot damage is much more common than true winter killing in hardy varieties of alfalfa in Alberta. Winter killing usually occurs only

#### *Alfalfa naturally infected with the winter crown rot pathogen.*

FIGS. 1, 2. Dead areas in severely damaged stands. FIG. 3. Initial killing in small patches. FIG. 4. Close view of dead patch. FIG. 5. Growth of dandelions and other weeds in dead areas. FIG. 6. Plants weakened or killed through rotting of the crown buds and underlying tissues. FIG. 7. Severe rotting of the crown and root. FIG. 8. Severely weakened old plant with partially proliferated crown. FIG. 9. Split root of old plant with completely rotted crown.





under exceptionally severe weather conditions when there is an ice sheet or insufficient snow cover, or after the plants have prematurely lost their hardened condition in the early spring. In winter killing all or most of the root system is affected, the root becomes soft and shredded, and the plant is easily pulled up. Plants partially injured as the result of adverse winter conditions have variable symptoms but lack the distinct crown lesions characteristic of winter crown rot (Figs. 6 to 9). Occasionally the symptoms of winter crown rot and true winter killing are found in the same field and even on the same plants.

Forage grasses attacked by the winter crown rot pathogen suffer damage similar to that caused by snow mold in turf grasses, described by Broadfoot (1). As in the cases of alfalfa and clover, the killing of timothy (Figs. 12, 13) creeping red fescue, and red top usually occurs in patches. Similar damage occurs in mixed stands of the legumes and grasses (Figs. 14 to 16). In some seasons extensive killing is also found in native pastures and meadows (Figs. 17, 18). These bare areas eventually fill in with annuals and quack grass or other rhizomatous plants.

## Distribution and Importance on Different Hosts

### ALFALFA

Winter crown rot has been found in all districts in which alfalfa seed is grown and also in many hay and pasture fields in central and northern Alberta and northern Saskatchewan. Particularly severe damage has occurred in the districts of Cherhill, Sangudo, Thorsby, and Westlock in west-central Alberta, and White Fox in northeastern Saskatchewan. In southern Alberta the disease occurs in many irrigated and nonirrigated stands of alfalfa, but causes relatively slight damage. The pathogen has also been isolated from diseased specimens sent from damaged stands in British Columbia and Manitoba. It has not yet been reported outside of Western Canada.

The severity of winter crown rot damage varies greatly from year to year. In west-central Alberta the general damage was estimated as moderate to severe in four out of six years (Table 1). The disease was most prevalent and severe in 1943, when 88% of the stands examined were infected and had an average estimated damage of 20%. Many stands were also ruined in 1941, 1942, and 1946, through killing of from 25 to 50% of the plants. In 1944 and 1945 there was relatively little damage, consisting mainly of a slight increase in size of the previously killed patches, or in the killing or weakening of individual plants or small patches (Fig. 3). This damage, however, undoubtedly helped in the incidence of severe killing again in 1946. During

*Legumes and grasses naturally infected with the winter crown rot pathogen.*

FIGS. 10 to 13. Dead areas in—10: alsike clover; 11: white Dutch clover; 12 and 13: timothy. FIGS. 14 to 16. Varying degrees of damage in mixed stands—14: alsike clover and timothy; 15: red top and alfalfa (left), brome grass and alfalfa (right); 16: creeping red fescue and alfalfa. FIGS. 17, 18. Dead areas in native pasture.

TABLE I

ESTIMATED DAMAGE CAUSED BY THE WINTER CROWN ROT PATHOGEN IN ALFALFA STANDS EXAMINED IN WEST-CENTRAL ALBERTA (1941-46)

Year	Percentage of stands* damaged					Total stands diseased, %	Average estimated damage in diseased stands, %
	None	Trace	Slight	Moderate	Severe		
1941	15	18	20	20	27	85	16
1942	36	12	18	16	18	64	15
1943	12	10	14	31	33	88	20
1944	67	9	22	2	—	33	3
1945	66	12	19	3	—	34	3
1946	28	3	21	23	25	72	18
Average for six years						62	10

\* Average of 120 stands examined each year.

the six year period the average estimated damage was 10%, with an average of 62% of the stands affected (Table I).

The amount of damage in different districts also varies greatly, apparently as a result of local environmental conditions during the winter. For example, the killing was particularly severe in northeastern Saskatchewan in 1942, and in west-central Alberta in 1943. As previously noted, infection is always less severe in the southern districts, where it results mainly in partial rotting of the crowns, with consequent weakening of the plants. This damage is not as important as the killing that occurs in the central and northern areas, but it probably contributes considerably to reduction in yield, as well as to shortening the life of the crop stands. In the irrigated areas winter crown rot commonly occurs in combination with the bacterial wilt disease.

Winter crown rot is most evident in relatively young, fairly thick alfalfa stands, where the pathogen apparently has the best opportunity to spread from plant to plant. It is usually most severe in stands three to four years old and seldom occurs in one-year-old stands, except in heavily infested land where a diseased crop has been recently ploughed up. The damage becomes less noticeable and may even cease after the plants become thinned out due to winter crown rot or other causes.

Observational evidence indicates that winter crown rot can be important under a wide range of soil conditions. It has been severe in alfalfa growing on both light and heavy soils in the black, transition, and gray wooded soil zones of Alberta. That damage is less in the brown soil zones of the southern districts is apparently explained by climatic conditions. In several instances the disease developed on alfalfa on new breaking and also following cereals and various other crops.

Observations made during the early season on losses due to winter crown rot may be quite misleading, since the damage cannot be accurately estimated

until late May or early June, when general growth is well advanced. Many plants that appear dead early in the spring may start late growth from crown buds that have escaped serious injury (Fig. 6). If the crown tissues are not too severely rotted and growth conditions are favorable, this weak late growth may give a fair yield of hay, especially on the second cut. Such growth is, however, usually too late for satisfactory seed production. Also, the weakened plants often do not survive the following winter.

### CLOVERS

Winter crown rot is frequently destructive in alsike clover, especially in the seed-growing areas of west-central Alberta. For example, it caused moderate to severe damage in 7 out of 17 fields examined in 1946. White or Dutch clover is also very susceptible and sometimes suffers severe damage along roadsides or when grown occasionally as a seed crop (Fig. 11). Vanterpool (9) found that white clover was killed out in snow mold patches in lawns at Saskatoon. Stands of red clover are often less severely affected, since the plants are usually killed individually rather than in patches. Red clover is less hardy than other clovers and alfalfa under Alberta conditions, and disease damage is sometimes confused with winter killing. Sweet clover suffers extremely severe damage when planted in land naturally or artificially infested with the pathogen.

### FORAGE GRASSES

The winter crown rot pathogen often causes damage in timothy, red top, and creeping red fescue in the central and northern districts. In 1946 severe damage was found in 9 out of 16 fields of timothy and in several mixed stands of timothy and alsike clover in west-central Alberta. The pathogen has also been isolated from naturally infected plants of meadow fescue and Kentucky bluegrass, but appears to be less prevalent on these hosts. It has seldom been found in brome grass, crested wheat grass, or slender wheat grass. Mixed stands consisting of alfalfa or clovers with susceptible grasses such as timothy, red top, or creeping red fescue usually suffer much more damage than those containing brome grass, which appear to be highly resistant (Figs. 14 to 16).

### OTHER HOSTS

The results of isolation and infection studies, not yet completed, indicate that the winter crown rot pathogen may cause damage in a wide range of cultivated and wild perennial plants. It has been isolated from naturally infected plants of the following cultivated species: *Iris germanica* L.; *Aconitum* sp.; *Phlox paniculata* L.; *Thymus Serpyllum* L.; and *Pastinaca sativa* L. Isolates have also been frequently obtained from diseased plants of *Taraxacum officinale* Weber, and *Agropyron repens* (L.) Beauv., and from the mycelium and dead plants of various species in snow mold patches in native pastures and meadows. The fungus was previously isolated by Broadfoot (1) from

severely damaged turf grasses at Edmonton, and by Vanterpool (9) from snow mold patches in lawns at Saskatoon. No damage has yet been found in plants growing in wooded land.

### Isolation Studies

Isolation of the winter crown rot pathogen is usually difficult, except from superficial mycelium or freshly infected host tissues obtained during the spring thaw. Later, the soil temperature starts to rise, and the diseased tissues are invaded by secondary parasites and saprophytes that often inhibit or retard growth of the pathogen, especially at temperatures above 10° C. Isolates are most easily obtained by plating fragments of mycelium from the surface of diseased tissues, and incubating them in a refrigerator at about 1° C. Potato dextrose agar is one of the most favorable media for isolation and growth of the pathogen. If superficial mycelium is not present, the fungus usually grows more readily from the base of the stems than from the crown or root tissues of diseased plants. Direct plating of the material without prior washing or surface sterilization generally gives the best results, since mercuric chloride and other fungicides tend to retard growth of the fungus.

Isolates of the pathogen grow slowly from plated mycelium or diseased tissues, and require about 10 to 14 days to produce observable growth on potato dextrose agar at 1° C. Once established, the whitish mycelium grows fairly rapidly in close contact with the medium, forming a zoned matted colony with straight radial growth of the hyphae clearly visible at the edge. White stroma-like structures are sometimes produced but no spores or sclerotia have been observed. The fungus can be identified microscopically by the numerous, typically basidiomycetous, clamp connections fully developed on the septate mycelium in colonies about two weeks old.

### Infection Studies

#### *Field Experiments*

Field infection of alfalfa and other hosts by the winter crown rot pathogen was studied by planting in infested land and by inoculation. Inoculations were made in the fall by applying inoculum grown on a soil-cornmeal medium or natural inoculum consisting of soil or sods taken from dead patches in fields or meadows. Individual plants were inoculated by placing a pinch of inoculum in the surface soil near the crowns and a similar method was used to start infection at marked points in rows or plots. In large plots or blocks of plants the inoculum was broadcast or spread thinly in a shallow trench near the rows. Since very extensive killing usually resulted from inoculation by the above method, many experiments were conducted in naturally and artificially infested soil.

Final notes on infection were taken after growth of the healthy plants was well started in the spring. In some experiments the dead plants were counted, and in others, the distance of spread of the killing in plots or rows was

measured. Plants that were damaged but not killed were given an infection rating based on the extent of rotting of the crown buds and tissues.

The crown and root symptoms resulting from inoculation or from planting in infested soil were similar to those observed under natural conditions (Figs. 6 to 9). Mycelial growth of the pathogen, however, was much more evident. It remained around the crowns of diseased alfalfa plants (Fig. 19) for a few days after the snow melted. This grayish-white, cobweb-like growth sometimes extended up the dead stems of the affected plants (Figs. 20, 21). In grasses and clovers the mycelium formed snow mold patches (Figs. 25, 26) similar to those found in turf grasses. The plants beneath the mycelium were usually killed. Large blocks of alfalfa were completely killed (Fig. 22) by inoculation or by planting in heavily infested land. When inoculum was placed at staked positions in the plots the plants were killed in patches (Fig. 24) similar to those occurring in naturally infested fields (Fig. 4). These patches enlarged and coalesced during successive years (Fig. 23).

#### *Crock Experiments*

Since it was not possible to make accurate observations on the early stages of field infection, attempts were made to study the disease under controlled conditions. Field-grown alfalfa plants were transplanted into 1-gal. crocks of soil in late October. In most experiments, the crocks, each containing 10 plants, were set in a shallow pit outside the greenhouse, with the rims at ground level and the intervening spaces filled with soil or peat.

Beginning in early November, two crocks of inoculated plants and a control were taken in at weekly intervals to study the progress of infection under various controlled conditions. These crocks were kept for a week at low temperatures to allow disease development and were then placed in the greenhouse. Final notes were taken when growth was well advanced in the control plants.

### **Factors Influencing Disease Development**

Attempts to explain the varying severity of winter crown rot damage from year to year and in different localities solely on the basis of general winter conditions have not been very successful. For example, severe damage occurred in many alfalfa fields in west-central Alberta in 1941, 1943, and 1946 (Table I), following winters that differed markedly in average air and soil temperature, snowfall, and other conditions. Other factors involving the pathogen and the host apparently interact with environmental conditions in influencing disease development in the late winter or early spring.

#### **GROWTH OF INOCULUM**

The results of inoculation studies indicate that disease development depends to some extent upon previous growth of the mycelium of the pathogen. In crock experiments, freshly grown inoculum placed in contact with susceptible plants required at least two weeks of undisturbed growth under favorable



conditions before it would cause infection. For example, in the 1944-45 experiment, infection of plants inoculated at various times and thawed at 2° C. in January was as follows:

Inoculated on Oct. 10.....	100%
Inoculated on Nov. 7.....	87%
Inoculated on Dec. 5.....	13%

Late inoculation apparently did not allow sufficient time for growth of the inoculum before freeze-up, since much more mycelium developed about the crowns of the plants inoculated in October than in December.

In field experiments, October inoculations invariably resulted in a higher degree of infection the following spring than those made after freeze-up in late November. No infection occurred when inoculations were made in the late winter or early spring. As in naturally infested soil, fall inoculation apparently affords the pathogen an opportunity to develop before conditions are optimum for infection in the late winter or early spring.

#### DORMANCY OF THE HOST

Under field conditions, winter crown rot damage always ceases while the snow is melting. Also, all attempts to secure infection of growing plants have failed. Evidence that the low-temperature basidiomycete is strictly a parasite of the dormant plants was confirmed in crock experiments conducted during seven winters, from 1940 to 1947, inclusive. Cocks of inoculated and control plants, removed from an outside pit at weekly intervals starting in early November, were held at about 2° C. for a week. No infection occurred in the first series of plants, even when there was an abundant growth of mycelium around the crowns. In subsequent series, disease development was at first restricted to the rotting of a few crown buds on each plant. As the season advanced the plants became progressively more susceptible (Table II and Figs. 27 to 30) until finally they were killed or greatly weakened (Fig. 30). The time when they first became susceptible to attack and the rate of increase in disease development varied considerably from year to year (Table II). The earliest and most rapid development occurred during 1943 to 1944, when the plants were first attacked in the series brought in on Nov. 17, and were completely susceptible in the Dec. 8 series. In contrast, during the winter of

#### *Winter crown rot development resulting from inoculation.*

FIG. 19. Mycelium of the pathogen in rows of dead alfalfa in early March. FIG. 20. Mycelium on alfalfa kept free of snow (top rows), and with normal snow cover (bottom row). FIG. 21. Mycelium on tops of dead alfalfa plants. FIG. 22. Plot of alfalfa completely killed by pathogen compared to noninoculated control plot at left. FIGS. 23 to 26. Dead patches produced as a result of placing inoculum at staked positions in the fall—23: extensive killing of alfalfa in successive seasons (below), two years after inoculation of healthy stand (above); 24: close view of artificially produced dead patch in alfalfa; 25, 26: mycelial patches in rows of clover in early March. FIGS. 27 to 30. Cocks of alfalfa plants brought inside and thawed at intervals in 1946-47—27, 28: Dec. 2 series, control and inoculated, respectively; 29: Dec. 16 series, inoculated; 30: Jan. 6 series, inoculated.

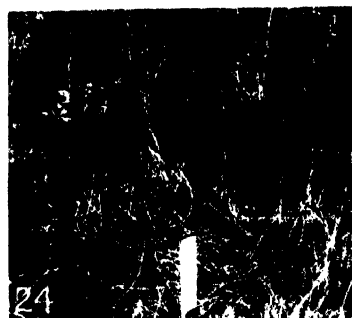




TABLE II

RELATION OF SOIL TEMPERATURE AND SNOW COVER TO THE TIME AND EXTENT OF WINTER CROWN ROT DEVELOPMENT IN INOCULATED ALFALFA PLANTS BROUGHT IN AT WEEKLY INTERVALS DURING THE EARLY WINTER (1941-47)

Winter	Disease development			Av. soil temp. <sup>a</sup> , ° F.		Snow cover	
	First	Moderate	Severe	Nov.	Dec.	Nov.	Dec.
1943-44	Nov. 17	Nov. 24	Dec. 8	30.4	30.2	None	Light
1945-46	Nov. 26	Dec. 3	Dec. 10	23.7	21.3	Light	Light
1940-41	Nov. 25	Dec. 9	Jan. 3	29.2	28.0	Light	Light
1941-42	Dec. 1	Dec. 15	Jan. 5	30.8	21.1	Light	Light
1946-47	Dec. 2	Dec. 16	Jan. 6	27.7	22.7	Light	Moderate
1942-43	Dec. 7	Dec. 21	Jan. 4	29.6	28.6	Moderate	Heavy
1944-45	Dec. 11	Dec. 26	Jan. 9	22.5	17.2	Moderate	Moderate

<sup>a</sup> Recorded at a depth of 3 in.

1944 to 1945 no infection occurred in any series until Dec. 11, and the damage was not severe until Jan. 9. These results indicate that susceptibility to attack by the pathogen is associated with the physiological changes accompanying dormancy and hardening in the host plant, and that the rate of these changes varies from year to year.

#### SOIL TEMPERATURE

Observations indicate that soil temperature does not have a direct effect on the degree of winter crown rot development. Severe damage occurred in 1942 and 1946 (Table I), when the average soil temperature during the winter was low (about 20° F.), and in 1941 and 1943, when it was relatively high (25° to 30° F.). In 1945 the damage was only slight after a winter during which the soil temperature dropped to 10° F.

Soil temperature appears to affect the development of root lesions. When the average soil temperature was relatively high and there was little frost in the ground, the root lesions often appeared for some distance below the crowns (Fig. 7). On the other hand, the lesions were usually confined to the crowns (Fig. 6) following winters when the ground was frozen hard.

The results of crock experiments (Table II) indicate that the time and rate at which the plants become susceptible to attack are not directly related to low soil temperatures. The average soil temperature was about 30° F. during November and December of 1943 to 1944, when the plants became most rapidly susceptible. Also, in other years there was no apparent correlation between soil temperatures and changes in host susceptibility. Freezing temperatures, however, proved necessary to bring about these changes. In several experiments, the plants in crocks placed close to the greenhouse, where radiation of heat delayed freezing, did not become susceptible so early as, and they suffered much less damage than, the plants in normally frozen soil.

## SOIL MOISTURE

The influence of soil moisture on disease development was studied by adding varying amounts of water to the soil in a series of crocks of inoculated plants. The moisture content of the soil could not be accurately controlled in the outside pit, but water was added when necessary to maintain the gradient between the different series. The average degree of infection obtained in a representative experiment (1944 to 1945) was as follows:

Dry soil series (about 35% m.h.c.).....	77%
Moist soil series (about 50% m.h.c.).....	60%
Wet soil series (about 70% m.h.c.).....	28%
Saturated soil series (about 90% m.h.c.).....	None

In all crock experiments there was decidedly less infection of the plants in wet or saturated soil than of those in dry or moist soil. Although the differences were less marked under field conditions, disease development was usually less severe in plots watered heavily in the late fall than in those not watered.

## SNOW COVER

Observations made over a period of years indicate that the amount of snow cover has much less influence on the development of winter crown rot than the time and manner in which it melts. The snow cover was variable in 1941, 1943, and 1946, when the most severe damage occurred (Table I), but in all three years it melted slowly, starting in late March or April. Winter crown rot damage in the experimental plots was also particularly severe in these three years and it was associated with an especially profuse growth of mycelium (Figs. 19 to 21). There was much less damage and also less mycelial growth in years when the snow melted early and rapidly. A similar relationship was observed in field experiments where one set of plots was completely cleared of snow prior to thawing in the early spring. A second set of plots was partially cleared at intervals during the winter, and a control set was left with an undisturbed snow cover. The average radial spread of the killing in these plots for a period of three years (1943 to 1946) was as follows:

Snow cleared before thawing.....	2.0 in.
Light snow cover.....	2.4 in.
Undisturbed snow cover.....	3.2 in.

Early removal of the snow apparently prevented maximum development of the pathogen, since there was much less mycelial growth in the cleared plots than in those left undisturbed.

The effect of temperature on the rate of thawing and disease development was studied in crock experiments. Crocks containing plants that had reached a susceptible stage were removed in late December from the outside pit described above and were thawed at controlled temperatures ranging from 1° to 20° C. They were then placed in the greenhouse until growth started

in the control plants. The average degree of infection obtained in a representative experiment (1945-46) was as follows:

Thawed in four days at 1° C.....	73%
Thawed in two days at 5° C.....	41%
Thawed in one day at 9° C.....	12%
Thawed in 12 hr. at 15° C.....	11%
Thawed in six hours at 20° C.....	None

In all experiments, slow thawing at a temperature near freezing favored disease development, regardless of the presence or amount of snow on the crocks.

Field studies also indicated that snow in itself was not essential for disease development, but merely provided favorable conditions. Replicated rows of inoculated alfalfa plants were kept free of snow throughout the winter by covering them with V-shaped wooden troughs with closed ends. These troughs were left on the rows until the snow melted in adjacent rows. Infection was as severe under the troughs as in the rows with a normal snow cover, and mycelium was abundant in both cases (Fig. 20).

The influence of snow cover on host dormancy and susceptibility in the late fall was also studied. As shown in Table II, the earliest and most rapid disease development occurred in years when there was little or no snow cover during November and December. In other winters the insulating effect of a heavier snowfall may have retarded the action of low temperatures on the physiological changes in the plants.

#### PRESENCE OF OTHER PATHOGENS

The association effects of other root and crown rotting fungi frequently isolated from plants primarily parasitized by the low-temperature basidiomycete were studied. In crock experiments, inoculum of the latter was mixed in equal proportions with that of each of several other pathogens, including *Cylindrocarpon Ehrenbergi* (4), *Plenodomus Meliloti* (7), *Sclerotinia sativa*, and *Fusarium avenaceum* (3). Complete killing of the plants occurred in all tests, but the presence of other pathogens, especially *C. Ehrenbergi* and *S. sativa*, resulted in more extensive rotting of the roots below the crown than that caused by the low-temperature basidiomycete alone. These results support the observation that certain root-rotting pathogens may sometimes increase the damage started by the winter crown rot pathogen.

#### Field Spread of the Pathogen

##### MANNER OF SPREAD

Aside from possible mechanical transfer by implements or other means, the winter crown rot pathogen appears to spread mainly by means of mycelium. No fruiting stage that would aid in its spread has yet been found in nature or

in artificial culture. Also, the extent and manner of spread of the fungus in the field do not indicate that spores are involved. For example, it did not spread across a 2 ft. path from infested experimental plots to control plots (Fig. 22), when reasonable precautions were taken.

It has been apparent that the fungus might spread both above and below ground, since infection was severe when the mycelium was abundant around the crowns (Figs. 19 to 21), and also sometimes when it was scarce or absent. In field experiments with alfalfa, various barriers were used in an attempt to determine the relative importance of these two types of spread. Replicated 6 in. pots, placed at intervals along the rows of plants, proved most suitable. For the aboveground series, inoculum was placed on the inside edge of the rim of pots nearly filled with soil. Each pot was buried so that the rim was at soil level and the pathogen had to pass over it and the intervening ground to reach the plants. To force spreading of the mycelium below ground, empty pots were inverted and inoculum was placed inside the slightly buried rims. In both series, three sets of pots were placed so that the inoculum was at distances of one-half, one, and two inches, respectively, from the crowns of the plants. Notes were taken in the spring on the extent of the killing in the row opposite each pot.

TABLE III

RELATIVE DEGREE OF KILLING OF ALFALFA PLANTS BY THE WINTER CROWN ROT PATHOGEN WHEN INOCULUM WAS PLACED IN DIFFERENT POSITIONS ABOVE- AND BELOW-GROUND. (FIELD EXPERIMENTS, 1944-45 AND 1945-46)

Position of inoculum	Distance from plants, in.	Av. number of plants killed	
		1944-45	1945-46
Aboveground, in pot buried at soil level	$\frac{1}{2}$	5.0	5.1
	1	4.8	2.3
	2	None	0.5
Below-ground inside inverted pot	$\frac{1}{2}$	5.0	5.5
	1	2.3	3.2
	2	0.5	1.3

As shown in Table III, killing of the plants resulted from spread of the mycelium above and below ground. Approximately the same number of plants were killed in both cases. No mycelium developed on the surface of the intervening soil in the below-ground series. Similar results were obtained in other experiments where the barriers and inoculum were placed so that the mycelium could spread from plant to plant in the rows.

The ability of the mycelium to spread upwards in the soil was studied by placing inoculum at depths ranging from 1 to 18 in. below the surface. Excavations were made at intervals along rows of alfalfa plants and at each staked

position a pinch of inoculum was carefully placed at the required depth in contact with the root of one plant. The average number of plants killed in a representative experiment (1942-43) was as follows:

Inoculum at surface.....	4.0
Inoculum at 1 in. depth.....	4.5
Inoculum at 3 in. depth.....	4.2
Inoculum at 6 in. depth.....	3.0
Inoculum at 9 in. depth.....	2.5
Inoculum at 12 in. depth.....	1.5
Inoculum at 15 in. depth.....	2.0
Inoculum at 18 in. depth.....	1.0

In this and other experiments killing took place over a smaller area with the deeper placement of the inoculum. However, even at the 18 in. depth, the plant in contact with the inoculum was usually killed. Severe rotting was not found at the point of inoculation on any of the roots in the above experiments. In all cases merely superficial lesions extended up the root to the crown, which was completely rotted. There was an apparent tendency for the mycelium to spread upwards through the soil in proximity to the roots and to cause injury only at the crown.

#### DISTANCE OF SPREAD

The distance of spread of the winter crown rot pathogen in alfalfa stands was determined by placing a small quantity of inoculum in the fall at staked positions in the center of plots or at intervals in rows of plants. Spread of the pathogen, as indicated by dead plants, was measured the following spring. As shown in Table IV, the average distance of spread of the killing varied considerably from year to year. The greatest spread (7.5 in.) occurred in 1942-43, when the damage in alfalfa fields was also particularly severe (Table I). It was least (2.7 in.) in 1943-44, when there was relatively slight damage in alfalfa fields.

TABLE IV

DISTANCE OF SPREAD OF THE WINTER CROWN ROT PATHOGEN AMONG ALFALFA PLANTS AND ACROSS BARE SOIL, AS INDICATED BY THE DEGREE OF KILLING (1942-46)

Winter	Average distance of spread of killing among plants, in.	Percentage of plants killed when inoculum was placed at various distances, in. from the rows							
		$\frac{1}{2}$	$\frac{1}{2}$	1	2	3	4	5	6
1942-43	7.5	100	100	98	92	72	72	52	36
1943-44	2.7	100	50	0	0	0	0	0	0
1944-45	3.0	100	84	80	80	8	0	0	0
1945-46	4.5	100	100	100	58	20	3	0	0
Average	4.4								



In studying the distance of spread of the pathogen across soil unoccupied by plants, inoculum was placed in shallow trenches parallel to rows of alfalfa at distances ranging from  $\frac{1}{2}$  to 6 in. Inoculations were made in the fall and the percentage of plants killed in each row was determined the following spring.

As shown in Table IV, the pathogen did not spread quite so readily across bare soil as among the plants in plots or rows. However, in the 1942-43 experiment there was killing in the rows 6 in. distant from the inoculum. The spread did not exceed  $\frac{1}{2}$  in. in 1943-44 and, as in the previous experiments, it was directly related to the severity of the damage in alfalfa fields.

### Discussion

The widespread occurrence of the winter crown rot pathogen on forage crops and other hosts in cultivated and virgin land in Alberta indicates that it has been long established, and may even be native in this region. Prior to the present study, the damage caused by this pathogen was probably attributed to true winter killing or to other crown- and root-rotting fungi. This is quite understandable since the damage occurs at about the same time as winter killing, and isolation of the pathogen is made difficult by secondary invasion of the tissues by other organisms. The pathogen undoubtedly increases under field conditions, and is becoming more destructive with the increasing cultivation of alfalfa and other highly susceptible crops. The limitations of mycelial spread, however, appear to prevent the pathogen from spreading to any extent from field to field. Little is yet known about the manner in which it persists and spreads in nature or in the absence of a susceptible host.

It is evident that there are several stages in the development of winter crown rot. In the first place, the pathogen apparently requires a period of mycelial growth under suitable conditions before freeze-up in the late fall. If infection does occur during the fall period it is probably not important, since the plants are not susceptible until they reach a certain stage of dormancy. By this time the soil is usually frozen and there is no opportunity for infection until thawing starts in the late winter or early spring. The pathogen then resumes growth and may spread very rapidly and cause severe damage if conditions are favorable. It develops best in the natural cavities formed at the soil surface beneath a slowly melting snow cover. This suggested cycle of development ends when the snow melts, since the plants become decreasingly susceptible as growth begins and they lose their hardened and dormant properties. As the soil temperature rises, the pathogen also ceases to develop, and becomes dormant until conditions are again favorable for growth during late fall.

Environmental conditions at the time of the first spring thaw have a marked effect on the nature of winter crown rot damage and on its severity from year to year and in different localities. Thawing first starts at the surface of the soil, enabling the pathogen to grow and attack the crowns of the plants. If the snow melts slowly, the crowns may be completely rotted and the plants killed while the underlying soil is still frozen. Present evidence is that early

and rapid melting of the snow invariably results in lessened damage. This may explain the relatively slight injury that occurs in southern Alberta, where the warm Chinook winds may cause rapid melting of the snow at any time during the winter. A similar relationship between a slowly melting snow covering and increased snow mold damage in grasses and cereals was observed by Remsberg and Hungerford (6), and Wernham and Chilton (10) in studies on *Typhula* spp., and by Schaffnit (8) with *Fusarium nivale*. Soil temperature and moisture and other factors also have an important influence on disease development in relation to host physiology and require further detailed study.

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# EFFECT OF CROP DEBRIS, PLANT ROOTS, AND CROP SEQUENCE ON THE MICROBIAL FLORA OF THE SOIL IN RELATION TO ROOT ROT IN CEREAL CROPS<sup>1</sup>

By L. E. TYNER<sup>2</sup>

## Abstract

The decomposition of wheat, oat, or barley straw in soil caused a marked increase in the microflora, as indicated by plate counts. The oat straw compost yielded significantly more colonies of fungi than either the wheat or barley straw composts. The population of bacteria and actinomycetes was increased to about the same degree by all three of the composts.

In another laboratory experiment, wheat, oats, barley, and beans were planted in various rotations in pots of soil from field plots known to be heavily infested with the root-rotting pathogens *Helminthosporium sativum* P. K. and B., *Ophiobolus graminis* Sacc., and *Fusarium* spp. Fungi, mostly *Penicillium* spp. and *Mucor* spp., were up to 15 times more abundant from the rhizosphere of wheat roots than from the rhizosphere of oat, barley, or bean roots, regardless of crop sequence. It is assumed that the higher counts of saprophytic fungi obtained from the rhizosphere of wheat seedlings were directly correlated with the greater amount of dead root tissue on this crop, since disease on the wheat seedlings was more severe than on the other hosts. In a duplicate experiment in fallow soil, the rhizospheres of wheat, oats, barley, and beans yielded about equal numbers of fungi.

## Introduction

In previous work (7), it was found that root rot on wheat seedlings was less in composts of soil with oat straw than with wheat or barley straw. It was thought that the microflora concerned in the decomposition of the oat straw depressed the root-rotting pathogens. In the present paper attempts were made to assess quantitatively the microflora in different straw-soil composts, and also to investigate the effects on it of both living and decomposing root systems of wheat, oats, barley, and beans.

## Methods and Results

### *Effect of Cereal Straw Upon the Growth of Microorganisms*

Finely chopped straw of wheat, of oats, and of barley was mixed with black soil to make 3% by weight of each mixture. The mixtures were then put into 1-gal. crocks; the moisture was adjusted to 20% content; and the crocks were covered with waxed paper and placed on a greenhouse bench. Two days afterwards, each crock was emptied, the compost thoroughly mixed, and a 100 gm. sample removed. The moisture content was again adjusted to 20% and the soil returned to the crocks. Samples were similarly removed every three weeks for seven successive periods.

Each 100 gm. sample was thoroughly mixed and a 40 gm. aliquot was taken; this was further subdivided into two 20 gm. portions, one of which was used

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to make dilutions. This portion was added to one liter of sterile tap water and shaken for five minutes. Ten milliliters was withdrawn and added to one liter of water; the mixture was well agitated. Of this concentration, 50 ml. was added to 150 ml. of water to make a dilution of 1 : 20,000 for counts of fungi, and 5 ml. was added to one liter of water to make a dilution of 1 : 1,000,000 for counts of bacteria and actinomycetes. In each case one milliliter of the final respective dilutions was added to seven replicated 10 cm. Petri plates and mixed with a synthetic agar medium for bacterial counts. When this medium was used for counts on fungi, it was supplemented with 0.25% boric acid to suppress bacterial growth (8). The plates were incubated at a temperature of 70° to 74° F. and the counts of fungi and of bacteria were made on the fourth and on the 10th day, respectively.

In addition to the use of soil-straw composts, dilutions were made in the above manner from crocks containing straw of each kind unmixed with soil. Counts of fungi and bacteria were made from these crocks on three different occasions, each at three-week intervals. All data appear in Table I.

TABLE I

NUMBER OF COLONIES OF FUNGI, BACTERIA, AND ACTINOMYCETES OBTAINED AT THREE-WEEK INTERVALS FROM DECOMPOSING STRAW AND 3% STRAW-SOIL COMPOSTS

Substrate	Fungi							Bacteria and actinomycetes						
	Thousands per gram							Millions per gram						
	Three-week intervals							Three-week intervals						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Wheat straw - soil	192	35	20	22	20	29	20	80	55	27	47	18	40	20
Oat straw - soil	272	320	178	90	84	146	69	71	84	43	45	16	43	15
Barley straw - soil	134	70	68	25	24	40	17	36	75	47	57	59	25	13
Soil	22	10	10	12	12	22	20	6	12	8	21	5	6	6
Wheat straw	272	312	137	—	—	—	—	70	53	19	—	—	—	—
Oat straw	1642	320	80	—	—	—	—	78	38	14	—	—	—	—
Barley straw	204	192	40	—	—	—	—	74	58	15	—	—	—	—

It will be noted that fungal colonies were most numerous in plates from dilutions of oat straw and oat straw composts. On the other hand, the number of colonies of bacteria seemed to be uninfluenced by the kind of straw. Colonies were always more numerous when straw was present in the soil than when it was absent, as in the controls. This indicates that the process of decomposition of plant debris is associated with a marked increase of both fungi and bacteria. The growth of fungi was greatly stimulated during the first three week period, and the growth of bacteria during the second period. Vandecaveye and Katznelson (9) also found that in the process of decomposition of organic matter the fungus group was more active than the bacterial

group in the initial stages of decomposition. The high numbers of fungi in oat straw or its compost suggest that this straw provided a more suitable medium for fungi than did wheat or barley straw.

*Influence of Previous Cropping Practice on the Microflora of the Rhizosphere of Various Field Crops*

Sufficient soil to fill 80 6-in. pots was taken in the fall from field plots that had been replanted continuously to wheat for 11 years, and that were known to carry a heavy natural infestation of root-rotting fungi. The 80 pots were separated into four groups of 18, with eight pots for controls.

In the first planting, 18 pots were planted to wheat, 18 to oats, 18 to barley, and 18 to beans, and the eight remaining control pots were left fallow. The pots were placed in large shallow trays on the greenhouse benches and watered from below to ensure a reasonable uniformity of moisture content. One month later, root-rot infection data were secured from the seedlings. The root systems were collected from all pots and freed of most of the adhering soil. Duplicate 10 gm. aliquots of the roots were weighed, one for making dilutions, and the other for computing on a dry basis the weight of roots and adhering soil. Ten grams of soil from the control pots were also taken for dilution. The dilutions were made from the 10 gm. aliquot of the roots that had been cut into segments of about 1 cm., and the plates were poured on the same day that the plants were lifted. The media and the incubation period were the same as in the last experiment. The numbers of fungi, actinomycetes, and bacteria were computed on a basis of the dry weight of the sample.

All pots were replanted on the day following the first harvest. The 18 pots that were planted to wheat in the first planting were replanted to wheat. Six of those planted to oats, barley, or beans, were planted to wheat, and the remaining 12 pots of each group were replanted to oats, barley, and beans, respectively. In the third planting all the 18 pots having wheat in the first and second plantings were replanted to wheat. Twelve of those having oats, barley, or beans were planted to wheat, and the remaining six pots in these groups were replanted to oats, barley, or beans, respectively. This planting sequence made it possible to secure data on the microflora of the rhizosphere from the following rotations: wheat, wheat, wheat; oats, wheat, wheat; oats, oats, wheat; oats, oats, oats; barley, wheat, wheat; barley, barley, wheat; barley, barley, barley; beans, wheat, wheat; beans, beans, wheat; beans, beans, beans; and the soil control. After the three plantings just described, the experiment was repeated, black Edmonton loam in fallow from the field being used instead of root-rot soil. All data obtained from this experiment, as well as those from the plantings in the root-rot soil, are shown graphically in Fig. 1.

Fig. 1 shows that very high numbers of fungus colonies were obtained from the rhizosphere of the roots of wheat plants grown in root-rot soil. Colonies were also very numerous from roots of wheat seedlings following an oat crop.

The increase was not so marked after barley or beans, and after two crops of beans there was apparently no increase on wheat roots. The average counts of fungi for the three plantings were 1550, 423, 146, 160, and 9 thousand per gram for wheat, oat, barley, and bean roots, and soil, respectively. The colonies from this soil comprised 64% *Mucor* spp., 29% *Penicillium* spp., and 7% miscellaneous spp. The disease rating varied directly with the observed trends in the fungus counts and was moderately high in all cases. This result was to be expected, as the soil in the field had been cropped to wheat for 11 years, which thereby favored an increase in wheat pathogens. On the other hand, the data indicated that organisms pathogenic to oats, barley, or beans were practically absent at the time of the first planting.

Average bacterial counts for the three plantings in root-rot soil were 183, 94, 264, 39, and 5 million per gram for wheat, oat, barley, bean roots, and soil, respectively. Bean and oat roots were much less favorable substrata for bacteria than barley or wheat roots. Except in the case of beans, replanting of the same crop favored increase in the bacterial population.

In the fallow soil the fungus colony counts were not excessively high, and there was a tendency for the count to decrease with successive plantings. The average numbers of colonies for three plantings were 235, 133, 178, 85, and 8 thousand per gram for wheat, oat, barley, and bean roots, and soil, respectively. The colonies comprised 48% *Mucor* spp., 46% *Penicillium* spp., and 6% miscellaneous spp. The contrast in counts between this soil and the root-rot soil was very marked in the case of wheat roots, but to a less extent in the case of oat roots.

TABLE II

INFECTION RATINGS OF WHEAT, OAT, AND BARLEY SEEDLINGS AFTER THREE SUCCESSIVE MONTHLY REPLANTINGS IN POTS OF SOIL FROM VARIOUS SOURCES

Soil sample	Infection, %								
	Wheat seedlings			Oat seedlings			Barley seedlings		
	Planting			Planting			Planting		
	1	2	3	1	2	3	1	2	3
After one oat crop	8	18	20	20	14	21	11	26	17
After one barley crop	25	12	23	17	22	28	15	11	16
After 12 wheat crops	25	23	33	4	9	19	4	21	23
Virgin soil	7	13	17	6	24	24	8	16	25
Under willow	2	4	8	6	17	15	6	22	20

The bacterial population was quite low in the fallow soil at the first planting, but the average counts for the three plantings were higher than those for the root-rot soil. The average number of colonies were 312, 204, 175, 134, and 2 million per gram for wheat, oat, barley, and bean roots, and soil, respectively. With the exception of two cases in which counts were unusually high on wheat roots, there was little difference in the population pattern for the different crops.

Infection ratings for seedlings grown in this fallow soil were low and averaged about the same for wheat, oats, and barley. It would appear that organisms pathogenic to the seedlings of these crops were not numerous in this soil.

It will be noted that fungi and bacteria were always more abundant in the rhizosphere of the seedlings than in the soil controls. Apparently the living roots stimulate the growth of microorganisms, and it is significant that the degree of stimulation may vary with the crops. Starkey (5), Clark (1), Timonin (6), Lochhead (2), and West and Lochhead (11) have reported on similar stimulations to microbial growth, in particular bacteria, from the presence of plant roots in the soil.

It seems that a complexity of factors influences the population of microorganisms in the rhizosphere. In the root-rot soil, conditions were very favorable for the growth of fungi on dead wheat roots, and probably the pathogens pave the way for other fungi, mainly *Penicillium* spp. and *Mucor* spp., by providing rotted tissue as food. In this connection data from a supplementary experiment showed that slightly over twice as many fungus and bacterial colonies developed from plated washings from the crowns and roots of moderately attacked wheat seedlings than from those containing only a trace of disease. None of these were root-rot pathogens. The bacterial colonies observed were 405 and 190 million per gram of dry root tissue, and the fungus colonies numbered 1.62 and 0.75 million per gram.

The number of fungus colonies obtained from the fallow soil was, by comparison, low, and did not increase with the replanting of the different crops. However, the bacterial population was relatively high in both fallow and root-rot soil, and it increased with replanting of the crops in both soils (Fig. 1). It may be that this effect of replanting on the bacteria is attributable to some growth stimulating substance left in the soil by the roots (6).

#### *Relative Degree of Infestation of Various Soils by Root-rotting Fungi*

It was decided to observe root-rot infestation as it might occur naturally in soils of the same type, but having different crop histories. Five such samples were secured for this purpose during October. One of these was obtained from a field plot that had just produced a crop of oats, another from a plot that had grown a crop of barley, and a third from a plot that had grown 12 continuous crops of wheat. The fourth sample was taken from unbroken virgin sod, and the fifth from under willow shrubs where no sod had formed. Four pots of each of these soil samples were planted with wheat, four with oats, and four with barley. The pots were placed in large shallow trays on greenhouse benches and watered from below. Data on infection were secured from three successive monthly plantings. The results appear in Table II.

The disease rating on wheat seedlings grown in soil after 12 crops of wheat was 25%, whereas the oat and barley seedlings were practically clean in the same soil. In the soil from oat stubble, the disease rating on the seedlings

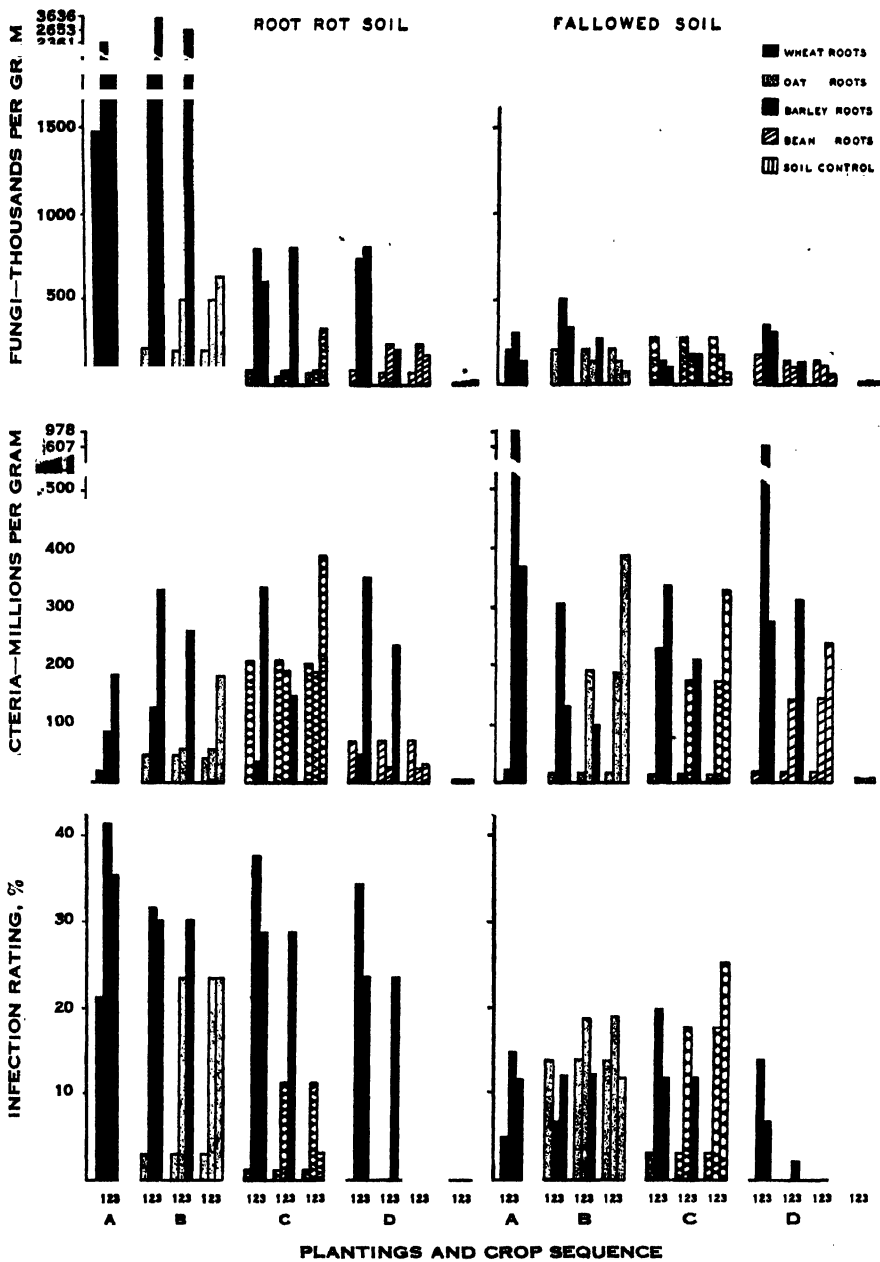


FIG. 1. Numbers of microorganisms in the rhizosphere of wheat, oat, barley, and bean seedlings from three successive monthly plantings in various rotations; and the infection-rating on the roots of the wheat, oat, and barley seedlings.

1, 2, 3 = plantings.

A, B, C, D = wheat; oats-wheat; barley-wheat; beans-wheat rotations, respectively.



was 20%, as compared to 8% and 11%, respectively, on wheat and barley seedlings grown in this soil. On the other hand, in the soil sample taken from barley stubble the disease rating was 15% on the barley seedlings, whereas it was 25% and 17% on wheat and oat seedlings, respectively.

No explanation is offered of the tendency for the disease rating gradually to increase on the wheat, barley, and oat seedlings in the second and third plantings in the willow and virgin soils. Possibly pathogenic *Fusarium* spp. and *Helminthosporium* spp. were indigenous, or the disinfected seed still carried inoculum, or air- and water-borne spores found access to the soil during the experiment.

### Discussion

A previous study (7) indicated that root rot on wheat seedlings grown in composts of soil with oat straw was consistently less than in composts with wheat or barley straw. In the present study it was found (Table I) that colonies of fungi, mainly *Penicillium* spp. and *Mucor* spp., were significantly more numerous in dilutions from decomposing oat straw or oat straw composts than in those from the wheat or barley straw or their composts. In view of these results it seems probable that in the previous study (7) the root-rot pathogens in the oat straw - soil compost were suppressed by the abundant development of such species in that compost. Sanford and Cormack (4) showed that *Penicillium* spp. were active in suppressing the pathogenicity of *Helminthosporium sativum*, and Sanford (3), in a review of the potential roles of association effects in plant disease, has indicated how pathogenicity may be accentuated or attenuated by the soil flora associations.

The present study has shown that fungus growth was greatly stimulated by the presence of roots, and, in particular, by wheat roots in a soil that had been cropped for 11 years to wheat in the field (Fig. 1). Infection ratings on the wheat seedlings grown in this soil were high, a result expected from the cropping practice mentioned. It might be considered anomalous that an abundant fungus growth in the oat straw compost (7) would reduce disease, whereas in the root-rot soil it would not. As already mentioned, bacteria, actinomycetes, and spores of certain fungi (*Penicillium* spp., and *Mucor* spp.) were greatly stimulated by the presence of roots. It is possible that the increase in the saprophytic fungi from the rhizosphere of wheat seedlings in the root-rot soil was a direct result of disease. Starkey (5) found by direct examination that microbial development was most extensive where dead root material was decomposing. Wheat roots even moderately infected with root rot show extensive lesions, not only in the region of the crown, but also at various places on the primary and secondary roots. Evidently this rotted tissue is a very favorable medium for the growth of fungi as well as bacteria. In general, there was a marked tendency for both microorganisms and root rot to increase with each successive planting of the same crop.

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# USE OF THE INFRARED TOTAL ABSORPTION METHOD FOR ESTIMATING THE TIME COURSE OF PHOTOSYNTHESIS AND TRANSPIRATION<sup>1</sup>

BY G. W. SCARTH,<sup>2</sup> A. LOEWY,<sup>3</sup> AND M. SHAW<sup>4</sup>

## Abstract

The type of apparatus designed by Dingle and Pryce for determining the concentration of carbon dioxide in air by non-spectroscopic measurement of its absorption of infrared has been modified so as to increase its accuracy greatly. Less than one part by volume of carbon dioxide in a million of air can now be measured at speeds ranging from 10-min.-interval to continuous readings. Water vapor can also be measured accurately and continuously.

Examples are given of the use of the technique in following the time course of photosynthesis and transpiration, including a correlation of the courses of these processes with leaf temperature and stomatal movement during rapid wilting of a detached leaf of *Pelargonium*.

## Introduction

Since carbon dioxide gas and water vapor absorb infrared and are the only constituents of normal air that do so, and since this affords a rapid and speedy means of estimating their concentration either in streaming or stationary air, it is rather surprising that so little use has been made of the technique in plant physiology. To the writers' knowledge the only application is that of McAlister (2) who developed it with great success to follow the time course of photosynthesis, including such transitory phenomena as 'induction', which appears for a few minutes at the start of a light period and 'dark pick up' of carbon dioxide, which occurs even more briefly after the light is cut off. McAlister's technique showed remarkable sensitivity as well as speed, measuring as low as one part of carbon dioxide to a million parts of air at intervals of a few seconds. The main drawback is the considerable complexity and cost of the apparatus required. To separate the absorption effect of carbon dioxide from that of water vapor he used an infrared spectroscope having rock salt optical parts. This allowed a narrow band of 4.2 to 4.3  $\mu$  wave lengths, which lies at the peak of the absorption band of carbon dioxide and outside that of water vapor, to be directed upon a vacuum thermocouple. The small current generated in the thermocouple required a sensitive galvanometer to measure it, and for certain purposes a photronic relay and secondary galvanometer in addition (3).

A simpler technique for measuring absorption by carbon dioxide was introduced by Dingle and Pryce (1). It dispenses with the spectroscope and requires less sensitivity in the detecting apparatus. With this method the

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carbon dioxide effect is isolated by drying the air before it enters the absorption chamber. Though Dingle and Pryce do not mention it, the method can also be applied to water vapor without even the necessity of removing carbon dioxide. Here the total absorption by the gas is measured, and a broad beam of radiation can be directed upon a thermopile. Hence less galvanometer sensitivity is needed. Another advantage is the relative independence of fluctuation in air temperature, which, while it alters the position of the absorption band, has little effect on the amount of absorption.

On the other hand, the absence of spectroscopic (or other) screening allows the thermopile to be heated by 'useless' radiation, which may be of much greater intensity than the part that is to be measured. Dingle and Pryce counteracted this effect by balancing their sampling absorption chamber and its thermopile against a similar control chamber and second thermopile. The differential current produced by the opposed e.m.f.'s is then determined by the difference in concentration of carbon dioxide (or water vapor) in the respective chambers. As a common source of infrared they used the flame of a Meker burner.

The only use of the apparatus that these authors report is in measuring the absorption by different concentrations of carbon dioxide made up for the purpose of constructing a calibration curve, and it is not clear whether the low galvanometer sensitivity they employed was determined by error in making up the supposed concentrations or in measuring them. If the latter, the apparatus had only 1/13 the sensitivity to carbon dioxide of McAlister's. Moreover, in measuring photosynthesis at the usual rate of air flow, which allows no more than one-fifth of the total carbon dioxide to be removed by the plant, the possible error when photosynthesis is at its maximum would be about 33%.

Since, however, there appeared to be no inherent reason why the total absorption method should be so greatly inferior in sensitivity to the spectroscopic one, we decided to investigate its possibilities. Starting with equipment essentially the same as Dingle and Pryce's, we found that its capacity to measure carbon dioxide was, in fact, no greater than the above estimate. However, by making various changes that, though not drastic individually, add up to produce an impressive total effect, we have increased stability to such an extent that a galvanometer sensitivity more than 20 times that used by Dingle and Pryce is profitable in making measurements. The apparatus is now capable of measuring carbon dioxide with even greater accuracy than McAlister's, though with less speed, and of measuring water vapor more accurately than is ever likely to be required. It has some obvious defects, most of which could be remedied easily in setting up new equipment, and it appears capable of much further development along different lines for particular purposes.

The present paper describes the apparatus as recently used by us and gives examples of the application of the technique to physiological studies. These comprise, as far as we know, the first reported physiological use of the total

absorption method, and the first application of any infrared absorption method to measurement of transpiration.

## The Apparatus

A description of the principal parts of the apparatus follows and a diagram of the whole assembly is shown in Fig. 1. It differs from the original form of the apparatus mainly in the source of infrared and in the sensitivity of the galvanometer.

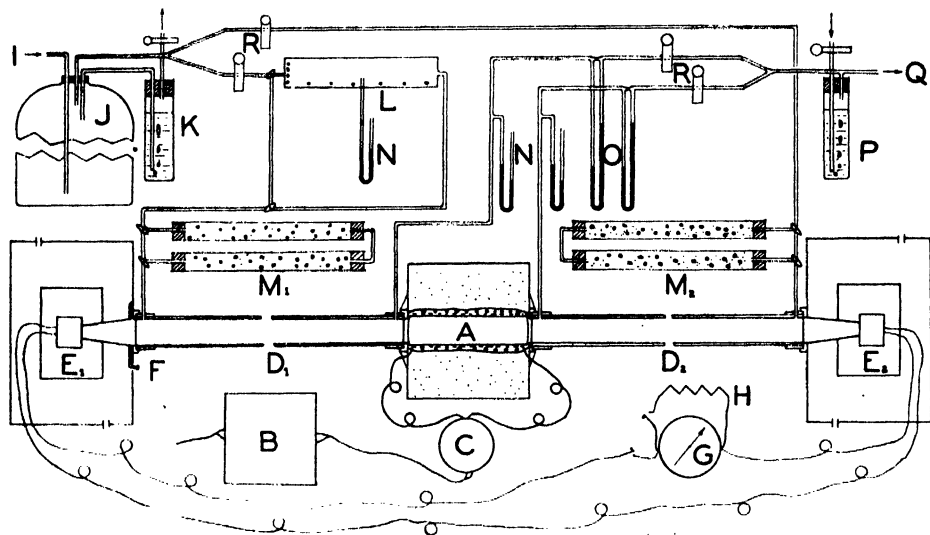


FIG. 1. *Diagram of the apparatus.*

- A — Heater, or infrared radiator, in median section.
- B — Constant voltage transformer.
- C — Variac.
- D<sub>1</sub> and D<sub>2</sub> — Infrared absorption tubes.
- E<sub>1</sub> and E<sub>2</sub> — Thermopiles.
- F — Iris diaphragm.
- G — Galvanometer.
- H — Damping resistance.
- I — Position of electric air pump.
- J — Air reservoir for regulating flow.
- K — Overflow valve.
- L — Leaf chamber.
- M<sub>1</sub> and M<sub>2</sub> — Drying tubes.
- N — Manometers.
- O — Flowmeters.
- P — Inlet valve.
- Q — Position of constant head suction pump.
- R — Adjustable clamps.

The *heater* (*A*), source of infrared, is an electrically heated quartz tube filled with carbon dioxide. The tube ( $18 \times 2.6$  cm.) is wrapped with 12 ft. of flat nichrome wire, having a resistance of 1.6 ohms per foot, and by asbestos string to hold the wire in place. This heater is enclosed by a couple of fire bricks and is set in alignment with the two absorption tubes. A collar of

asbestos cement covers the junction with the latter. With the aid of a constant voltage transformer (input load 100 to 120 v., 7.5 to 12 amp.; output 115 v., 1.75 amp.) and a variac, the heater is supplied with a very steady current. This current of 65 v. and 3 amp. produces a temperature of about 800° C. at the middle of the quartz tube.

The *infrared absorption tubes* ( $D_1$  and  $D_2$ ) are of stainless steel, polished inside, 190 cm. in length and 2.56 cm. internal diameter. Their ends are fitted with brass sleeves and screw-on caps that hold mica windows in place. As there is considerable absorption of useful wave lengths by mica, the sheets are made only thick enough to withstand buckling under the highest pressure difference that is likely to be set up, about 20 cm. of oil. Near each end of the absorption tubes small lateral tubes for inlet or outlet of air are inserted.

The *thermopiles* ( $E_1$  and  $E_2$ ) are the Moll small surface type, which has 16 thermocouples. Their conical reflectors just fit the ends of the absorption tubes. The sensitivity of the two thermopiles was found to be unequal, in the ratio of 6 to 5. They are therefore exposed to radiation intensities of approximately inverse ratio. An iris diaphragm ( $F$ ) interposed in front of one of them facilitates the balancing. Each thermopile is enclosed in a copper box and this in a larger cardboard one through which air can circulate. The use of reflectors to concentrate the rays has this advantage over the use of larger thermopiles that it does not amplify the effect of changes in external air temperature.

The *galvanometer* ( $G$ ) is a Cambridge moving magnet one, shielded pattern, resistance 22 ohms. Its maximum sensitivity, about 4000 mm. per  $\mu$  a. at 1 meter, and even the catalogued sensitivity of 1200 mm. is far beyond requirement, but the sensitivity can be varied without damping. For photosynthesis measurement sensitivities ranging from 500 to 650 mm. per  $\mu$  a. are found to be convenient, for transpiration less than 1/10th of that. In changing briefly from one type of measurement to the other sensitivity was reduced by damping rather than setting the galvanometer.

*Support* for all parts of the apparatus is firm and steady. The galvanometer is set on a concrete pillar resting on the concrete floor of a basement room. The heater, absorption tubes, and thermopiles, after being aligned for maximum sensitivity, were securely fixed to a rigid wooden base. This not only prevents accidental displacement of parts but allows the whole to be moved as one piece.

The *air stream system* is represented in the diagram in a somewhat condensed form. Fresh air is brought in from outside the building by means of an electric pump at  $I$ . After passing a pressure regulator (and also certain air conditioning devices not included in the diagram) the stream divides. Each branch in its course travels through, or may by-pass, a set of drying tubes ( $M$ ) filled with magnesium and barium perchlorates, then through one of the absorption tubes ( $D$ ), past a manometer, and through a flowmeter. In addition one branch can be routed through the leaf chamber ( $L$ ) situated

anterior to the drying tubes. The stream, united again beyond the flowmeters, passes through another regulator and is drawn off by a constant head suction pump at  $Q$ .

The rate of flow and pressure gradient in the common stream are roughly adjusted by varying the openings in the overflow valve ( $K$ ) and inlet valve ( $P$ ) and are more precisely adjusted in the branches by means of two pairs of hinge clamps ( $R$ ) that embrace sections of pressure tubing. This multiplication of controls is due to the necessity of maintaining the leaf chamber at atmospheric pressure and also equalizing the pressure in the two absorption tubes at any desired rate of flow.

The *leaf chamber* ( $L$ ) consists of a Petri dish lid resting on a glass plate, the edge being sealed with plasticine when the leaf is in place. The leaf used in the experiments described later was that of *Pelargonium zonale*. A notch in the edge of the lid accommodates the petiole and admits the wires of a thermocouple. Fitted into the lid are inlet, outlet, and manometer tubes. A wire grid supports the leaf and a gauze baffle at the inlet distributes the air evenly. It is not necessary to describe here the means whereby temperature and humidity are controlled, but it may be mentioned that the measurement of environmental conditions is a subsidiary use of the apparatus. It provides a rapid and easy means of measuring the vapor pressure in the air stream, its galvanometer connected with thermocouples gives the temperature of the leaf or air and in conjunction with a photoelectric cell reads the light intensity at the level of the leaf. Thus the operator can observe at will on his galvanometer scale the course of photosynthesis, transpiration, or respiration and the environmental conditions that normally control the rate of these processes.

### Calibration

In calibrating the apparatus for carbon dioxide measurement 'normal' dried air was run through the control absorption tube, and normal air mixed with carbon dioxide free air (both dried) in known proportions through the

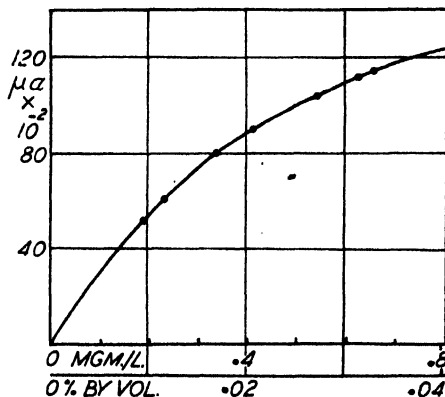


FIG. 2. Galvanometer current vs. carbon dioxide content of the air.

'experimental' tube. The carbon dioxide content of the normal air was measured by absorption in baryta solution and titration. The mixing was done with the aid of carefully calibrated flowmeters. In plotting the calibration curve (Fig. 2), the deflection obtained with carbon dioxide free air was

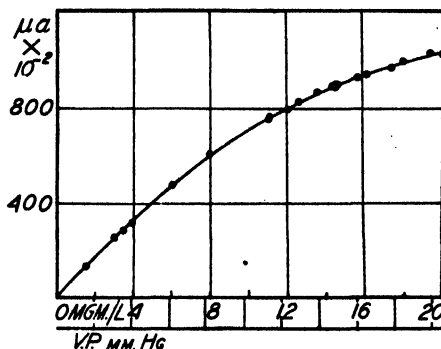


FIG. 3. Galvanometer current vs. moisture content of the air.

made the zero point and deflection is converted to current flowing through the galvanometer. This allows the curve to be used for any galvanometer sensitivity and any concentration of atmospheric carbon dioxide with a minimum of calculation.

In calibrating for water vapor most of the vapor pressures used were those of normal air on different days, the values being calculated from sling psychrometer measurements. Vapor pressures below 8 mm. Hg were obtained by mixing normal and dried air. The measurements of galvanometer deflection were made with dried air as a control. The calibration curve (Fig. 3) shows the current produced by different moisture contents expressed both as vapor pressure in mm. of Hg and as milligrams of water per liter of air at N.T.P.

### Operation and Performance

After the heater is turned on and air stream set in motion about 45 min. has to elapse before equilibrium is reached. Then, with the same air flowing through both absorption tubes, the galvanometer deflection, or 'apparatus zero' as we shall call it, is noted. This commonly fluctuates narrowly and slowly about the galvanometer zero, but occasionally it shows a protracted, steady one way drift—the result of a recognizable asymmetrical action of radiation or air temperature on the thermopiles. The fluctuating type of zero shift is negligible at the low sensitivities used for measuring transpiration, but for accurate measurement of photosynthesis it usually renders necessary the determination of apparatus zero alternately with experimental readings. During periods of fairly constant room temperature, however, satisfactory continuous records of photosynthesis are obtained even at high sensitivities.

The balancing for purpose of zero determination has to be done with the same kind of air in the tubes as that which occupies the control tube during



measurement, that is, dried air for photosynthesis and normal air for transpiration. During the course of measurement of photosynthesis the zero is checked by switching the air past the leaf chamber for long enough time to flush out the measuring tube completely. It is often more convenient to use the respiration level as a base line, darkening the leaf for a few minutes.

The usual type of fluctuation now encountered is illustrated in Fig. 4 where the irregularity in the photosynthesis record is known from other measure-

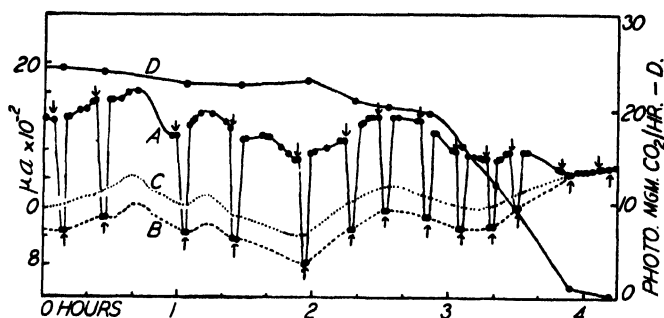


FIG. 4. Photosynthesis rate of a wilting leaf vs. time: type of record obtained with an average degree of zero fluctuation.

Curve A — Galvanometer current with leaf exposed to the light.

B — Galvanometer current with leaf darkened, leaf temperature unchanged: respiration level.

C — Probable apparatus zero level estimated from B and respiration rate.

D — Rate of gross photosynthesis of the leaf (right hand scale), calculated from Curves A, B, and from Fig. 2.

ments to correspond to that of the apparatus zero. Fig. 6 illustrates a relatively steady record with only one swerve (in the dark period) that can be ascribed to zero shift.

As an indication of the sensitivity of the apparatus to carbon dioxide, the current generated by 'normal' air (.03% carbon dioxide) vs. carbon dioxide free air (both dried) is  $1.10 \mu a$ . With the galvanometer set so that this current produces 650 mm. deflection at 1 meter, individual readings can be made accurately to within 1 mm. and readings taken before and after zero determination, if not identical, do not differ more than 1 mm. as a rule. The average of the two measurements, therefore, is likely to be accurate to within 1 to 2 mm. representing a concentration of  $0.5$  to  $1 \times 10^{-4} \%$  carbon dioxide. In general, a variation of less than one part of carbon dioxide per million (by volume) can be detected in normal air.

In measuring photosynthesis or transpiration by any gas stream method the rate of air flow is a factor. The error in measuring the latter is probably greater than in measuring concentration of gas by infrared absorption and will affect the accuracy of calculated *absolute* rates of either process. But, as regards *relative* rates, the rate of flow is so steady that little error is introduced by assuming it to be constant.

The *speed* of measurement as applied to photosynthesis is commonly limited by the time consumed in determining the apparatus zero between readings—about six minutes with the air stream flowing at 40 liters per hour.

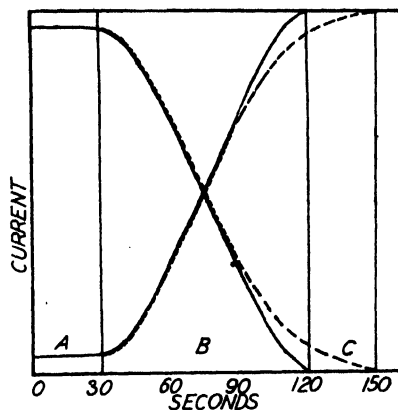


FIG. 5. Apparatus lag in recording a sudden change in moisture content of the air made at zero on time scale. Flow 40 liters per hour.

Continuous curve—Change made at outlet of leaf chamber.

Broken curve—Change made at inlet of leaf chamber.

Period A — Initial or transit lag.

B — Absorption tube lag.

C — Leaf chamber lag.

When the only zero determination, if any, necessary is that of the galvanometer, true speed of measurement is still limited by the time required for complete flushing out of the 'experimental' tube, because changes in the composition of the air occurring in less than this length of time are, as McAlister puts it, integrated or 'smeared out' in measurement.

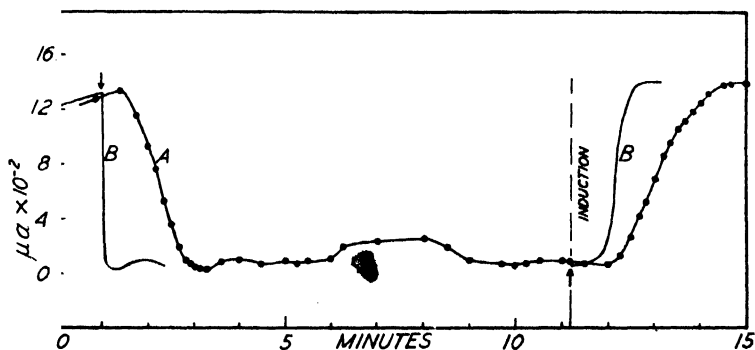


FIG. 6. Course of photosynthesis following a sudden change from light to dark and from dark to light; ↑ light out, ↓ light on.

Curve A — Galvanometer current vs. time as recorded.

B — Portions of Curve A corrected for apparatus lag.

It is possible, however, to correct for lag and 'spread' produced by the apparatus itself. Fig. 5 shows the course of galvanometer deflection that followed an abrupt change in the composition of the air produced (a) at the exit and (b) at the entrance of the leaf chamber. There is an initial lag of 30 sec. before any effect appears, the time required for the air to travel from the leaf chamber to the absorption tube. The only effect of this lag on measurement is to delay it. The slope that follows represents the progressive displacement of one type of air by the other in the absorption tube. The continuous curve flattens out about 90 sec. after it begins to fall, which is practically the time required to empty this tube as calculated on the basis of volume. The broken curve 'tail' extending over a further 30 sec. or so represents the emptying of the leaf chamber, delayed because of its shape by the formation of turbulent eddies. This sloping curve presents not only a lag but also a 'spread' of the event, lasting only a few seconds, which it records. Applied, however, to correct the curve of photosynthesis in Fig. 6 it allows a truer one of the time course to be drawn. The corrected curve shows clearly the 'induction' period that follows the start of illumination and indicates also that uptake of carbon dioxide does not cease abruptly on darkening.

While it is stretching the speed capacity of the apparatus in its present form to use it for the resolution of such rapid changes as are indicated in the previous experiment, it is admirably adapted for following those that are naturally timed in minutes rather than seconds. As an example, Fig. 7 shows what has probably never been measured before, the correlated time courses of transpiration, leaf temperature, photosynthesis, and stomatal movement during the rapid wilting of a leaf that, while exposed to a stream of dry air, was deprived of its water supply by cutting of the petiole. Two experiments, made on adjoining leaves of the same plant, are recorded here. In one, transpiration and leaf temperature were measured concurrently and in addition small strips were torn from the edge of the leaf at intervals for absolute alcohol fixation and stomatal measurement. Under constant external conditions the time curves of transpiration and leaf temperature always form mirror images of one another when plotted on an appropriate scale, so that leaf temperature can be used as an indirect measure of transpiration. Accordingly photosynthesis and leaf temperature were followed together in the second experiment.

Comparing the curves in Fig. 7A, we see that transpiration and stomatal opening increase slightly for a few minutes after the petiole is cut. (Passive opening of stomata at incipient wilting has frequently been recorded by other methods.) Then, for a time, transpiration drops more rapidly than stomatal closure, indicating some non-stomatal form of internal control. Later the two curves follow a parallel course of decline, and transpiration flattens quickly into the cuticular rate at the moment that stomatal closure is completed.

The photosynthesis curve in Fig. 7B shows a brief lag but no rise in its response to cutting. The decline that follows is rapid and parallels stomatal closure. At the completion of closure net photosynthesis is zero and gradually thereafter gross photosynthesis falls below the rate of respiration. To

correlate the two experiments, the inflections in the respective leaf temperature curves, which mark completion of closure, are synchronized in Fig. 7C. It appears that drop in rate of photosynthesis takes the lead of stomatal closure.

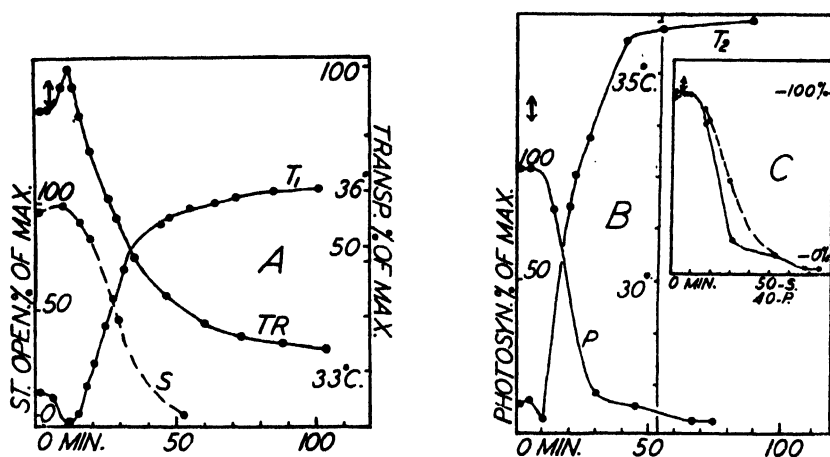


FIG. 7. Changes during rapid wilting of a leaf; ↑ petiole cut.

Tr — transpiration; S — stomatal aperture; P — photosynthesis;  $T_1$  and  $T_2$  — leaf temperature.

Figs. A and B are separate experiments. Fig. C shows S from A and P from B plotted on different time scales so as to make the inflections of  $T_1$  and  $T_2$  (closure of stomata completed) synchronize.

The final 'exhibit' selected from our records of time courses is apparently a manifestation of physiological rhythm. While the records of transpiration and photosynthesis in Fig. 8 are not concurrent and do not apply to the same leaf, we have evidence that when one of the processes fluctuates in this way the other does so too. In each of the records shown here the light was turned on at its start and during the rest of the period external conditions remained practically constant. Owing to the relative infrequency of the photosynthesis readings the exact shape of the lower curve is uncertain but, as in other records of this type, the position of the points is at least compatible with as smooth and regular fluctuation as is clearly demonstrated for transpiration. This particular record of photosynthesis was chosen because it shows the oscillation fading out and reviving vigorously under uniform external conditions. In other cases a change of light intensity has induced renewal of oscillation after its complete cessation. On the basis of our present experience the occurrence of the phenomenon is quite unpredictable. Though not common neither can it be called rare. No doubt the oscillations in stomatal movement that have been reported are another aspect of the same phenomenon, but where the basic rhythm is seated has yet to be determined.

The apparatus is useful, and has been used, for quite another kind of purpose than the above set of examples. It serves to escape the effect of time rather than to observe it when applied to measuring transpiration or

photosynthesis under varied conditions before any appreciable stomatal change has had time to occur. But it seems unnecessary to multiply examples further to demonstrate the value of the technique.

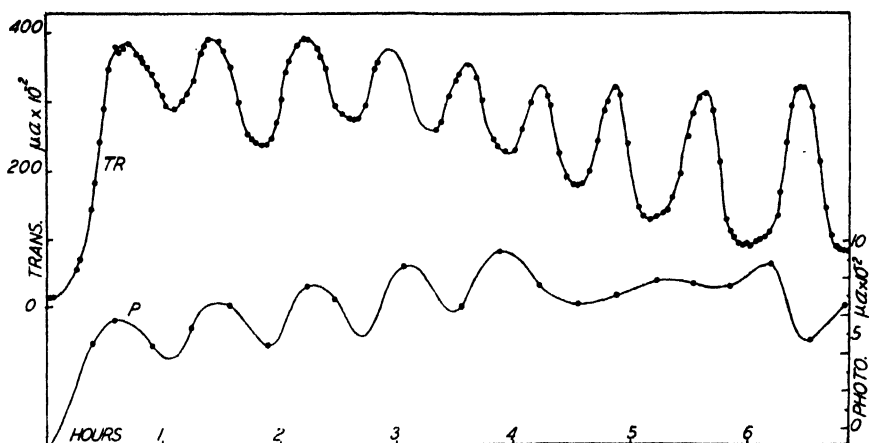


FIG. 8. Rhythmic fluctuation in transpiration ( $Tr$ ) and photosynthesis ( $P$ ) under constant external conditions.  
The two curves are not directly related, being obtained with different leaves and at different times.

### Defects and Their Remedy

Certain obvious defects that we have not yet had the opportunity to correct should be pointed out.

A fault of general design is the wide separation of the thermopiles, which renders them very liable to be subjected to differential changes in air temperature. This is found to be one of the most important causes of drift in apparatus zero. A better arrangement of parts would be to place the absorption tubes and thermopiles side by side, enclosing the latter in the same box. The present type of heater could easily be adjusted to suit the change by giving a U shape to the quartz tube.

Another defect, due to accident rather than design, is the asymmetry of the apparatus as shown by shifting of the zero when air temperature changes symmetrically with respect to the thermopiles or when a switch is made from normal to dry air in both tubes. The main cause of this asymmetry, at least in relation to external factors, is no doubt the marked inequality of sensitivity shown by our thermopiles. Another cause of the upset of balance that is produced by a change of air in the tubes may be a difference in the proportion of the various wave lengths emitted by the respective ends of the heater. As no precaution was taken to make the quartz of equal thickness at these points, asymmetry with respect to quality of radiation is not unlikely.

In the quality of this radiation, too, there is much room for improvement. As compared with the Meker burner, the heater has about one-third of the efficiency in producing wave lengths absorbed by carbon dioxide and three or

four times the efficiency in those absorbed by water vapor. But the reverse ratio would be more nearly ideal for the dual purpose of measuring both photosynthesis and transpiration. Whether due mainly to the weakness of the hot carbon dioxide source or to the relative opacity of quartz to wave lengths above  $4\ \mu$ , much of the radiation, as we have found, comes from the surface of the quartz itself. (Hence the need for shielding the ends of the tube from air currents.) Improvement in the present type of heater might be effected by giving it thinner end windows of equal thickness.

Finally, on more theoretical grounds it is suggested that the efficiency of the apparatus would be increased by reducing the ratio of length to width of the absorption tubes—though the optimum volume will depend on the balance of speed and accuracy of measurement demanded for various types of investigation.

The relation of absorption to length of tube with carbon dioxide concentration constant must be similar to that of absorption (galvanometer current) to the amount of carbon dioxide (or water) in a tube of fixed length. The latter is shown, of course, in our calibration curve—which incidentally Dr. Rowles has found to be of exponential shape (cf. Fig. 9). If there were total reflection

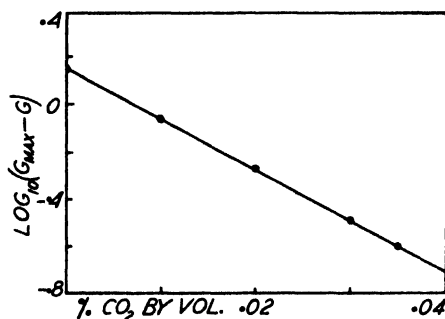


FIG. 9. *Logarithmic plot of carbon dioxide calibration curve, Fig. 2.  $G_{MAX} = 1.43\ \mu a$ . galvanometer current, to which the calibration curve is asymptotic.  $G$  = galvanometer current as in Fig. 2.*

inside the tube it would seem that the respective curves should be practically identical and equivalent to that of the absorption of a parallel beam by different thicknesses of medium. Taking the carbon dioxide calibration curve, therefore, as representing this hypothetical case of identity, and substituting percentage of present tube length for concentration of carbon dioxide (.03% carbon dioxide = 100% length), the galvanometer current produced by removing *all* the carbon dioxide from one tube is shown on the graph. As used for measuring photosynthesis, however, a more useful criterion of sensitivity in relation to tube length is the effect of removing a small proportion, say 1/10th, of the atmospheric carbon dioxide. This too can be estimated from the calibration curve. Some relative values are shown in the following Table I.

Under actual conditions the deflection percentages in the shorter tubes should be somewhat higher even, as this calculation assumes that no radiation is lost to the walls of the tube in transit.

TABLE I  
APPROXIMATE RELATION BETWEEN GALVANOMETER  
DEFLECTION AND TUBE LENGTH IN  
PERCENTAGE OF PRESENT VALUES

Length of tube	Deflection with all CO <sub>2</sub> removed	Deflection with 1/10 of CO <sub>2</sub> removed
100	100	100
50	67	90
25	40	70

The most efficient way, therefore, of increasing speed of measurement is to reduce the volume of the absorption tubes by shortening them. Any other method involves a loss in sensitivity proportional to the gain in speed.

Moreover, for the many purposes for which the present speed of measurement (with zero determination eliminated) is adequate, greater sensitivity to carbon dioxide can be obtained by widening the tubes in proportion as they are shortened. Assuming that the diameter of the heater is increased to fit, that its ends still emit the same intensity of radiation per unit area, and that the thermopile reflectors are enlarged to collect the full width of the 'beam', tubes of one-quarter the present length and four times the cross sectional area would increase sensitivity to small changes in carbon dioxide concentration 2.8 ( $4 \times 0.7$ ) times without any increase in sensitivity to external temperature changes.

No doubt further improvement could be made by more radical departures from the present design, but this is a subject for the physicist and outside the scope of this discussion.

### Conclusion

The infrared total absorption technique as now improved, and still more as likely to be further improved by such changes as have been indicated, possesses unique value for studying the time course of photosynthesis and transpiration, the influence of external factors on the rate of these processes uncomplicated by change in stomatal aperture, and other relationships requiring speed of measurement for their estimation.

As the equipment is not very expensive, nor its manipulation difficult, the method seems worthy of widespread adoption.

### Acknowledgments

Grateful acknowledgment is due to Prof. W. Rowles of Macdonald College for the loan of apparatus and for much valuable advice; also to the Department of Entomology, Macdonald College, for the use of laboratory space.

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## CHROMOSOME BREAKAGE INDUCED BY ABSORBED RADIOACTIVE PHOSPHORUS, $P^{32}$ <sup>1</sup>

By T. J. ARNASON,<sup>2</sup> ELAINE CUMMING,<sup>3</sup> AND J. W. T. SPINKS<sup>4</sup>

### Abstract

Radioactive phosphorus ( $P^{32}$ ) was made available to individual germinating seeds and seedlings of *Triticum vulgare*, *T. durum*, *T. monococcum*, and *Hordeum distichon*. Each treated plant was provided with either 0.18 or 0.018 microcuries of  $P^{32}$ . Both concentrations were effective in causing chromosome breakage and rearrangements. Many aberrant chromosome configurations occurred in microsporocytes of treated tetraploid and hexaploid plants. Only one chromosome aberration was found in *T. monococcum* and none in barley. Structural changes occurred also in chromosomes of *vulgare* wheat grown in soil to which fertilizer containing  $P^{32}$  was added. It is possible that the effectiveness of small amounts of  $P^{32}$  in inducing mutations is increased by its inclusion in chromosome molecules. Emitted beta particles would then be shot off very close to the sensitive target. Further, the recoiling atomic nucleus is almost certain to have enough energy to break any chemical bond. In any case since there is a change in atomic number (from 15 to 16) and in valence (P valence 5, S valence 2), molecular bonds must be released in the process.

### Introduction

An investigation of the effectiveness of absorbed radioactive elements in inducing mutations in plants was begun in the spring of 1947. A search of the literature yielded no reports on that topic. This was somewhat surprising since a considerable mass of information has been assembled regarding the effectiveness of beta, gamma, and other rays, emitted from an external source, in inducing chromosome breakage and gene mutation (3). By the use of appropriate radioactive elements it is possible to introduce a source of beta or gamma radiation into the living cells themselves and even into the parts known to be of especial importance in heredity: the chromosomes. As will be pointed out in the discussion, some special effects may be expected if the radioactive atoms are in the gene molecules or in the molecules making up the 'backbone' of a chromatid.

The use of absorbed radioisotopes has certain undesirable features, namely: (1) Exact dosage is not very easily controlled and may require rather tedious procedures to determine exactly. (2) It is not possible to stop the dosage at any desired time. (3) Constant vigilance must be exercised to prevent

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contamination of ground, instruments, personnel, etc. (4) The preparation, purification, and standardization of the appropriate solutions involves painstaking and repetitious labor. However, for certain types of work, e.g., treating large numbers of plants just before flowering, it may be more convenient to use solutions of radioactive elements than to use other radiation methods.

Radioactive phosphorus  $P^{32}$  was used since it appeared to be particularly well suited for experiments of this kind. Phosphorus salts are readily absorbed by plants and apparently there is free movement of phosphorus within plants (2). This element is a constituent of nucleoproteins, which are abundant in chromosomes and probably in genes (6). Consequently we can expect that some of the disintegrating atoms will actually be those incorporated in the chromosomes, possibly also in the genes.

### Materials and Methods

Plant species used as test organisms included the following: *Triticum vulgare* Vill. variety Thatcher ( $n=21$ ), *Triticum durum* Desf. variety Pelissier ( $n=14$ ), *Triticum monococcum* L. ( $n=7$ ), and *Hordeum distichon* L. variety Hannchen ( $n=7$ ).

Seed germination tests (7) had proved that seeds of these species could germinate individually in test tubes containing 0.1 ml. of nutrient solution including as much as 0.18  $\mu$ c. (microcuries) of  $P^{32}$  in the form of disodium hydrogen phosphate. Twenty-four seeds of each species were germinated in the 0.18  $\mu$ c. solution, another 24 in 0.018  $\mu$ c. solution, and six in nutrient solution with no active phosphorus. When the solution was absorbed, Knop's nutrient solution with phosphorus omitted was added to each test tube. Measurement of the amount of  $P^{32}$  remaining in the test tubes at the end of 13 days indicated that  $90 \pm 4\%$  of the active phosphorus had been absorbed by the plants. At this time the seedlings were transferred to 1-gal. crocks containing untreated soil.

When the plants had reached the appropriate stage (about six weeks after seed germination) anthers were collected and fixed with 3 : 1 alcohol-acetic mixture. Microsporocytes were smeared in iron-acetocarmine, the preparations being made permanent by mounting in diaphane.

Pollen mother cells were also obtained from Thatcher wheat plants grown in soil to which had been added 9.6  $\mu$ c. of  $P^{32}$  in the form of disodium hydrogen phosphate for each plant. The cells were then examined to determine whether any chromosome aberrations were present.

### Results

A summary of the treatments given and the numbers and chief kinds of chromosome rearrangements observed in microsporocytes is provided in Table I. The following aberrant chromosome configurations were seen: (1) at diakinesis and metaphase I—fragments, chains of three and of four chromosomes, rings of four, and univalents (Figs. 1 and 3); (2) at anaphase

TABLE I

NUMBERS OF MICROSPOROCTES HAVING VISIBLE CHROMOSOME ABERRATIONS FOLLOWING SEEDLING ABSORPTION OF  $P^{32}$ 

Species	Number of plants	Microcuries of $P^{32}$ per plant	Number of cells counted	Number of cells aberrant	Number of 'block mutations'	Common rearrangements
<i>Triticum monococcum</i>	6	0	65	0	0	Inversion
	24	0.018	170	26	1	
	24	0.18	112	0	0	
<i>Triticum durum</i>	6	0	54	0	0	Intrachromosomal rearrangements Inversion; fused sister chromatids
	24	0.018	215	23	3	
	24	0.18	172	30	4	
<i>Triticum vulgare</i>	6	0	73	1	0	Chiasma not formed Inversion; fragmentation Fragmentation; translocation; inversion
	24	0.018	160	9	2	
	24	0.18	230	24	6	
<i>Hordeum distichon</i>	6	0	164	0	0	
	24	0.018	72	0	0	
	24	0.18	115	0	0	

and telophase I—split univalents (Figs. 2, 5, and 6) acentric fragments (Figs. 4 and 11), bridges; (3) at anaphase II—bridges (Fig. 9), lagging chromosomes, and fragments; (4) at telophase II and in young microspores—extra-nuclear chromatin or micronuclei (Figs. 7, 8, 10).

In at least 15 anthers several or many cells had similar or related configurations indicating that chromosome breakage and rearrangement had occurred some cell generations earlier. Such groups of cells are here designated as having a block mutation. These mutations have proved their capacity to survive the mitotic cycle and some of them are likely to be capable of hereditary transmission through spores and gametophytes. It appears reasonable to suppose that chromosome breakage occurred with relatively high frequency in seedlings, when the  $P^{32}$  activity was at a maximum and the plant volume relatively small. However, since in some heads particular chromosome rearrangements occurred in only one or two anthers, of several examined, and since single aberrant cells were sometimes found among normal appearing ones it is suggested that breakage and reunion of broken ends continued up to the time when the material was gathered for the cytological investigation. We recognize, however, that small internal chromosome rearrangements might result in noticeable cytological peculiarities in a fraction only of the affected cells.

It is of some interest to note that the two diploid species yielded only one block mutation whereas in the tetraploid durum and in the hexaploid vulgare seven and eight such mutations were found respectively. It may be that

TABLE II

CHROMOSOME ABERRATIONS IN MICROSPOROCYTES OF THATCHER WHEAT SUPPLIED WITH  $P^{32}$  IN THE FORM OF  $Na_2HPO_4$  FERTILIZER ADDED TO THE SOIL

Anther number	Meiosis stages	Number of cells counted	Cells visibly aberrant, %	Types of aberrant configurations	Interpretation and conclusion
1	Meta., Ana. I*	64	6.2	Ana. bridge, fragment	Inversion
2	Meta. I	37	2.7	Chain of 3	Translocation and loss of chromosome
3	Diak., Meta. I	32	12.5	Ring of 4	Reciprocal translocation
4	Meta., Ana. I	40	2.5	Bridge and fragment	Inversion
5	Meta., Ana. I	34	0.0	—	—
6	Meta., Ana. I	60	83.3	1-10 univ. (split at anaphase)	Block mutation. Probably intrachromosomal rearrangements
7	Meta., Ana. II	65	0.0	—	—
8	Meta.-Telo. II	75	0.0	—	—
9	Telo.-Tetrads II	41	0.0	—	—
10	Meta. I	60	1.7	Chain of 3	Translocation and loss of one chromosome
11	Meta. I	51	84.3	20 pairs and 1 long univ.	Block mutation. One chromosome lost
12	Ana. II	55	0.0	—	—
13	Ana. I	11	0.0	—	No rearrangement
14	Meta. I	22	9.1	2 unequal univ. 1 univ.	Deficiency
15	Meta. I	18	11.1	Ring of 4	Reciprocal translocation
16	Diak.-Meta. I	28	92.9	Chain of 4 I univ. off plate	Block mutation. One chromosome lost
17	Meta.-Telo. II	64	0.0	—	—
18	Telo. I, Meta. II	19	0.0	—	—
19	Diak. I	10	0.0	—	—

\* I refers to first meiotic division.

FIGS. 1 TO 11. Photomicrographs of wheat microsporocytes and spores. FIGS. 1 TO 6: *T. vulgare*. FIGS. 7 TO 10: *T. durum*. FIG. 11: *T. monococcum*. FIGS. 1 AND 3: metaphase I in anther number 11, Table II. One univalent at left end. 475 $\times$ . FIG. 2: anaphase I in anther 16 of Table II. Split univalent between chromosome groups. 485 $\times$ . FIG. 4: early telophase I. Split fragment. (0.18  $\mu$ c.  $P^{32}$ ). 460 $\times$ . FIG. 5: anaphase I in anther number 6 of Table II. Approximately eight univalents split. 470 $\times$ . FIG. 6: anaphase I. Two split univalents. (0.18  $\mu$ c.  $P^{32}$ ). 440 $\times$ . FIG. 7: telophase II. Broken bridge (0.18  $\mu$ c.  $P^{32}$ ). 450 $\times$ . FIG. 8: tetrad. Three dense chromatin masses outside nuclei. (0.18  $\mu$ c.  $P^{32}$ ). 480 $\times$ . FIG. 9: telophase II. Chromosome bridge. (0.18  $\mu$ c.  $P^{32}$ ). 600 $\times$ . FIG. 10: telophase II. Split fragments. (0.018  $\mu$ c.  $P^{32}$ ). 425 $\times$ . FIG. 11: anaphase I. Lagging acentric fragments. (0.018  $\mu$ c.  $P^{32}$ ). 460 $\times$ .





the greater total chromosome length in the polyploid nuclei has simply provided more chances for breakage to occur or it may be that many kinds of rearrangement are cell lethal in diploids but not in polyploids, e.g., a homozygous deficiency may be fatal to diploid but not to polyploid cells since, in the latter, genes similar to those lost are present in other chromosomes.

Of the 19 anthers examined from the Thatcher wheat plants grown in soil to which  $P^{32}$  had been added, six had second division figures and tetrads only. Several types of rearrangements might escape detection at these stages. Block mutations were found in three of the remaining anthers. In two of these (Figs. 1, 2, 3) one chromosome had been lost; in the third (Fig. 5) there was a large but varying number of unpaired chromosomes (up to 10). Possible causes of this failure to pair include extensive intrachromosomal rearrangements and mutation or loss of a gene influencing pairing. A summary of the cytological results is provided in Table II.

The cytological collections were carried on for three days. On the day following the last collection the plants were harvested, dried, weighed, and ashed; counts were then made of the amount of active  $P^{32}$  present. Roots were not included. A complete set of anthers was collected from one plant. The mass of these anthers (air-dried) was determined to be 0.0035 gm. The average mass (dry weight) of a plant as harvested was 1.25 gm. The total amount of activity in an average plant was 0.04  $\mu$ c. In the tested anther set the total amount of active  $P^{32}$  was 0.0023  $\mu$ c. Based on these figures the mass of the anthers is estimated at 0.28% of the mass of the whole plant; the  $P^{32}$  present in the anthers is a much larger part of the whole, being 5.7% of the total activity in the plant.

### Discussion

The preliminary results reported in this paper and previously mentioned in a brief note in *Science* (1) indicate that small doses of  $P^{32}$  absorbed by young plants and present in meristematic cells are effective in causing numerous chromosome breaks. Only a small fraction of the breaks induced in apical meristems of the plants are likely to be represented and identifiable in microsporocytes. Breakage having cell lethal effects and breakages in cells not in the direct cell lineage of anthers will not be found. Small changes are likely to be overlooked. The effectiveness of small amounts of absorbed  $P^{32}$  may be ascribed in part to the fact that phosphorus is a constituent of many proteins and in particular of nucleoproteins, which are abundant in chromosomes (6) and probably in genes. Beta particles that are emitted by atoms located in the gene molecule or elsewhere in chromosomes must have, because of the closeness of the firing point to the target, a higher probability of passing through gene molecules or the protein backbone (5) threads of chromatids than beta particles emitted from a more distant source. Furthermore, the recoiling atom nucleus is almost certain to have sufficient energy to break any chemical bond (4). According to Lea (3) 4 ev. of energy is sufficient to dissociate the C—H bond in a molecule. A change in atomic number



occurs when  $P^{32}$  emits an electron,  $^{32}_{15}P$  becoming  $^{32}_{16}S$  with accompanying change in valence from 5 to 2. It is apparent that in any case the new atom cannot maintain all the bonds joining to the original P atom. Some of the molecular bonds involving  $P^{32}$  must break when the radioactive transformation occurs. The occurrence of these phenomena may make radioactive substances such as  $P^{32}$ , which may be incorporated in gene or chromosome molecules, highly effective in producing chromosome breaks and possibly potent in inducing gene mutations.

### Acknowledgments

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# TETRAPLOID *TARAXACUM KOK-SAGHYZ*

## IV. COMPARISON OF SECOND GENERATION FAMILIES<sup>1</sup>

By M. W. BANNAN<sup>2</sup>

### Abstract

The general similarity in root weight between the populations from reciprocal crosses of tetraploids, the differences between unrelated families, and the divergence between the progeny of large-rooted and small-rooted parents together indicate the operation of genetic factors in root development. Number, size, and shape of leaves likewise appear to be strongly influenced by heredity. As a rule, vigorous rosettes, large roots, and a sparsity or absence of first-year flowering are associated, but diverse assortments of these characteristics occur in some families. Since sparse flowering favors root enlargement, the later blooming and less floriferous tetraploids have an advantage over diploids in that respect. On the whole, random tetraploids possess wider but markedly fewer leaves, slightly smaller leaf area, bloom later and much more sparsely during the first year, and produce larger and more robust roots than diploids.

### Introduction

Although kok-saghyz is poorly adapted for genetical studies because of its incompatibility mechanism, the potentialities of the species as a rubber producer have rendered important the acquiring of information on the heritability of such characteristics as root size and rubber content. While diploids are more suitable for such studies than tetraploids, extensive experimentation with both has not been possible, and since the writer's investigations have been concerned mainly with tetraploids, these were used for crossing. First generation tetraploids derived from colchicine-treated parents were selected for root size, segregated into two groups, and the largest and smallest crossed among themselves to yield a second filial generation. The various morphological characters of the families in this second generation are described and comparison made both with each other and, in the matter of root size, with the parents.

### Methods

After removal from pots in the autumn of 1945 the roots of first generation tetraploids were weighed, the largest and smallest repotted and sunk in the plot for overwintering. Early the following spring the plants were removed to the greenhouse and the largest and smallest crossed among themselves. As flowering waned the roots were once again weighed and the rubber content determined. From the seed lots so obtained selections were made on the basis of parental root size and rubber content, some of the best and poorest combinations being chosen. The achenes, which were stored during the winter of 1946-47 at room temperature, were sorted in April 1947, the empty hulls removed, and the apparently sound specimens weighed.

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Contribution from the Department of Botany, University of Toronto, Toronto, Ont. with assistance from a grant in aid of research furnished by the University of Toronto.

<sup>2</sup> Assistant Professor in Botany.

These were placed on moist filter paper in Petri dishes without pregermination chilling. When the radicles protruded, the seedlings were set out singly in sterile soil in  $2\frac{1}{2}$  in. pots. In late May and early June the seedlings were transplanted in the garden plot at 7 in. spacings in rows alternately 12 and 18 in. apart, the maximum spacing in the limited area available. As the plants grew, records were kept of the number of leaves, diameter of the rosette, and head production for all plants, and estimates made of the seasonal march of leaf area for plants representative of the different families. In early October the roots were carefully removed from the soil, washed, and weighed, the weights including the defoliated crowns.

Control diploids were grown from mixed achenes harvested in 1946. One-half the plants were allowed to flower and produce achenes, and the remainder were disbudded. In the hope of determining the effect of age of achenes on plant development, parallel series of tetraploids and diploids were grown from mixed 1945 achenes, and two lots of diploids from 1945 and 1944 achenes. Unfortunately, for reasons beyond control, these lots were of different origin. The 1944 achenes were collected from open-pollinated plants grown locally; the 1945 achenes were provided by Dr. L. Truscott of the Ontario Agricultural College; and those of 1946 were gathered from open-pollinated plants originating from the 1945 achenes. Possibly because of genetic variability associated with difference of origin, these series failed to provide information of comparative value.

### Rosette Characters

Data on the number of leaves and diameter of the rosette at maximum size are given in Table I for various  $4n$  and  $2n$  series. In the tetraploids the biggest rosettes were usually found among the descendants of large-rooted parents, though considerable interfamily diversity occurred in this group,  $1690 \times 2076$  possessing rosettes more than twice as leafy as  $2073 \times 1845$ . Families of small-rooted lineage, such as  $2062 \times 2057$ , tended to have less vigorous rosettes, the diameter especially being noticeably reduced. On the whole, however, the differences in rosette size between derivatives of large or small plants were not as great as the differences in root size. Perhaps the most striking feature of the data in Table I is the general similarity in rosette size between the reciprocal crosses. For instance, in the case of  $2073 \times 2050$  and  $2050 \times 2073$  (two crosses) the average number of leaves at the maximum was 79.1, 79.6, and 80.9 and the average diameter 13.9, 14.1, and 14.7 in. The similarities between the reciprocal crosses, which stand in contrast to the interfamily and intergroup diversity, may be interpreted as indicative of genetic influence on rosette development.

The seasonal march of leaf area is illustrated in Fig. 1 for approximately 90 plants representing nine crosses. Leaf area followed a generally parallel course for each cross and its reciprocal, but differed considerably in the various families. In this connection it should be explained that the early separation of the families into two groups, as shown in the figure, was due to slight

TABLE I

DATA ON FIRST-YEAR, TETRAPLOID FAMILIES AND DIPLOIDS FROM MIXED ACHENES, GROWN IN THE GARDEN PLOT (1947)

Description	Av. root weight parents, gm.	Av. weight achenes, mgm.	Germ-ination, %	No. of plants	Av. no. of leaves	Predom-inant leaf type	Av. diam. rosette, in.	Av. no. of heads	Fresh root weight, gm.
4n 2073 × 2061	64.7	0.92	67	8	66.0	IV-V	17.8	24.8	57.1
2061 × 2073	64.7	0.56	66	32	56.0	IV-V	15.5	10.9	46.5
2073 × 1845a	64.5	0.77	44	14	49.4	IV	13.9	1.5	40.1
2073 × 1845b	64.5	0.80	67	26	48.6	IV-V	13.1	3.1	49.1
1845 × 2073	64.5	0.68	18	6	51.0	IV	12.6	7.0	32.0
2056 × 2073a	63.0	0.63	79	25	74.0	IV-V	13.9	12.9	49.7
2056 × 2073b	63.0	0.41	81	30	67.5	IV	13.2	12.0	43.1
2073 × 2050	57.5	0.89	97	27	79.1	IV	13.9	4.1	50.8
2050 × 2073a	57.5	0.44	77	39	79.6	IV	14.1	5.3	48.2
2050 × 2073b	57.5	0.49	79	36	80.9	IV	14.7	11.8	47.8
2056 × 2076	43.0	0.50	92	26	79.5	III-IV	16.8	11.2	30.3
2050 × 1845	42.2	0.54	35	11	59.0	III-IV	13.8	3.2	35.6
1845 × 2050	42.2	0.81	57	26	77.0	III-IV	14.2	16.0	41.7
2056 × 2050	41.5	0.54	90	46	80.6	IV	12.8	8.8	37.7
2061 × 1690	40.5	0.62	71	25	67.2	IV	13.8	5.1	45.1
2050 × 2076	37.7	0.61	55	21	76.6	III	16.5	12.1	34.2
2076 × 2050	37.7	0.81	93	23	78.4	III	16.8	27.5	28.0
2076 × 1690	35.2	0.86	94	23	86.0	IV	18.4	11.3	41.7
1690 × 2076	35.2	0.59	81	12	123.8	IV	16.7	1.5	49.3
4n 1780 × 2057	9.5	0.52	60	19	78.2	III-IV	15.7	23.5	31.7
2062 × 2057	9.0	0.66	41	9	51.9	III-IV	8.5	0	18.3
2069 × 1614	8.7	0.70	85	29	51.0	III-IV	12.1	4.4	26.1
1641 × 2062	8.7	0.76	41	17	73.5	III-IV	12.5	6.8	34.8
2062 × 1641	8.7	0.63	56	9	61.9	IV	11.1	0	26.7
4n mixed 2-yr. achenes	—	0.72	88	39	63.0	—	12.8	17.9	35.9
2n mixed 1-yr. achenes (diabudded)	—	0.48	94	35	92.3	—	11.2	87.1	15.0
2n mixed 1-yr. achenes (flowering)	—	0.48	94	39	71.6	—	10.8	45.1	12.7
2n mixed 2-yr. achenes	—	0.41	66	57	116.6	—	12.6	88.4	24.8
2n mixed 3-yr. achenes	—	0.41	35	17	133.0	—	11.6	25.6	37.7

differences in greenhouse illumination and time of transplantation outside. After transplanting, all plants grew rapidly and, in the case of the families investigated, leaf area expanded at more or less similar rates. The feature of particular significance when contrasting the families is the divergence after the attainment of maximum size. It will be observed that the reciprocals 2050 × 2076 and 2076 × 2050 conformed to the usual pattern for kok-saghyz, leaf area falling off sharply during the rest period in late summer and early autumn. In contrast the rosettes of 2073 × 2050 and reciprocal underwent continuous replenishment so that leaf area remained steady until harvesting. Scarth *et al.* (17), in their studies on summer dormancy in diploid kok-saghyz, came to the conclusion that the growth cycles in kok-saghyz were due to an internal rhythm. They believed that a setback in rosette size took place at a definite stage in ontogeny and this recession could be modified but not pre-

vented by environmental manipulation. The observations on tetraploids recorded in the present paper indicate the possibility of family differences in the rhythm of growth that would appear to have a genetic basis.

Among the random diploids, those grown from three-year-old achenes had the leafiest rosettes and the derivatives of one-year-old achenes the smallest

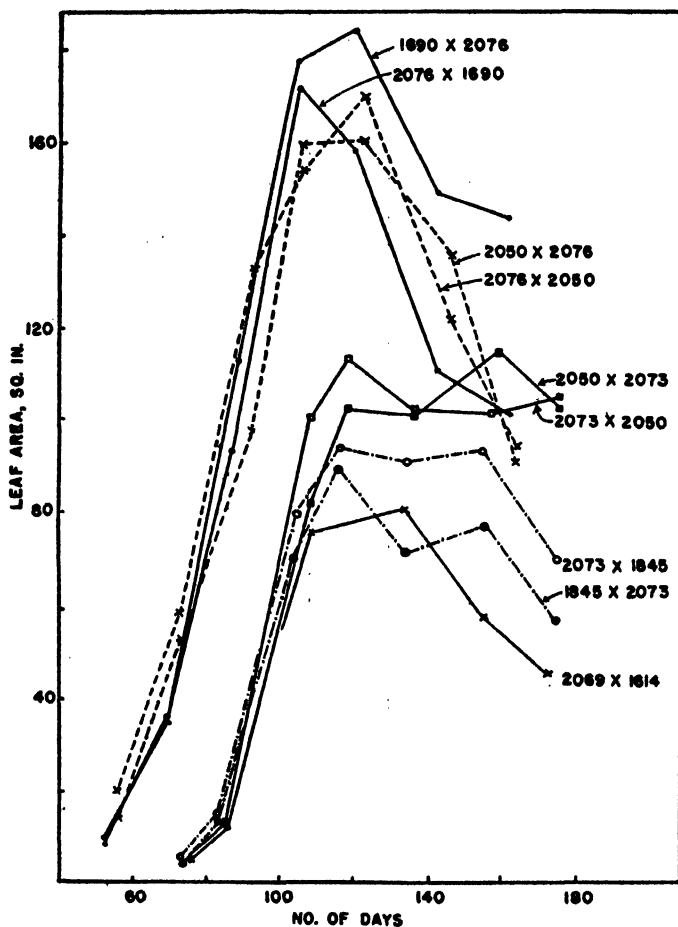


FIG. 1. Seasonal march of leaf area in representative  $4n$  reciprocal crosses.

(Table I). In the latter, disbudded plants produced leafier and wider rosettes than those allowed to flower, but the size in both disbudded and flowering plants was unaccountably small. In the parallel series of tetraploids and diploids grown from mixed two-year-old achenes, the latter had 72% more leaves than the former. This profusion of leaves in the diploids more than compensated for the small size of individual leaves, so that at maximum development leaf area slightly exceeded that of comparable tetraploids (Fig. 2). A similar slight superiority among diploids was reported in a previous

paper (3) for potted plants. The late-summer recession, however, was less drastic in the tetraploids.

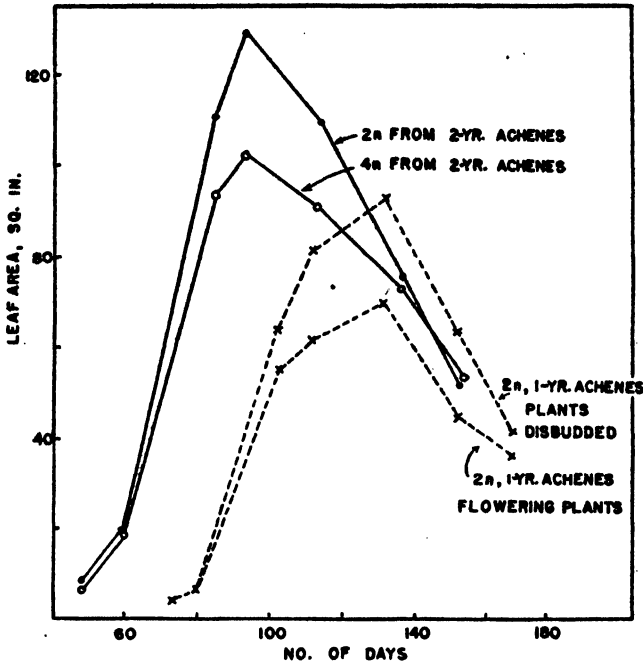


FIG. 2. Seasonal alterations in leaf area in diploids and tetraploids grown from mixed two-year-old achenes, and disbudded and flowering diploids from one-year-old achenes.

### Root Size

As shown in Table I the progeny of large-rooted tetraploids developed robust roots, although there was in most cases some regression from the parental size. Conversely the descendants of small-rooted plants produced roots below average size, although superior to the parents. The average root weights in the two groups were quite distinct, being  $42.0 \pm 7.7$  gm. for the progeny of large plants and  $26.7 \pm 6.3$  gm. for the offspring of small plants. The root weights differed in various lots, but it is noteworthy that they were generally similar in the cross and its reciprocal. For instance, in  $2050 \times 2073$  (two crosses) and the reciprocal  $2073 \times 2050$  the average weights were 47.8, 48.2, and 50.8 gm. The greatest difference occurred in  $1845 \times 2073$ , where the average weight was only 32 gm. as contrasted to 40.1 and 49.1 gm. for the reciprocals. Here, however, special circumstances existed. The achenes of  $1845 \times 2073$  germinated more poorly than in any other lot, and few seedlings developed. As these neared maturity their numbers were further reduced by rot, the percentage loss being greater than in other groups and much exceeding the 1% loss for the plot as a whole. As a consequence of these unfortunate developments only six plants were harvested, a number much too small for

the data to have significance. On the whole, the results from the various crosses point to hereditary influence on root size.

The root habit of the tetraploids was highly variable (Fig. 3). Some possessed stout tap roots with only feeble secondaries. Others had coarse

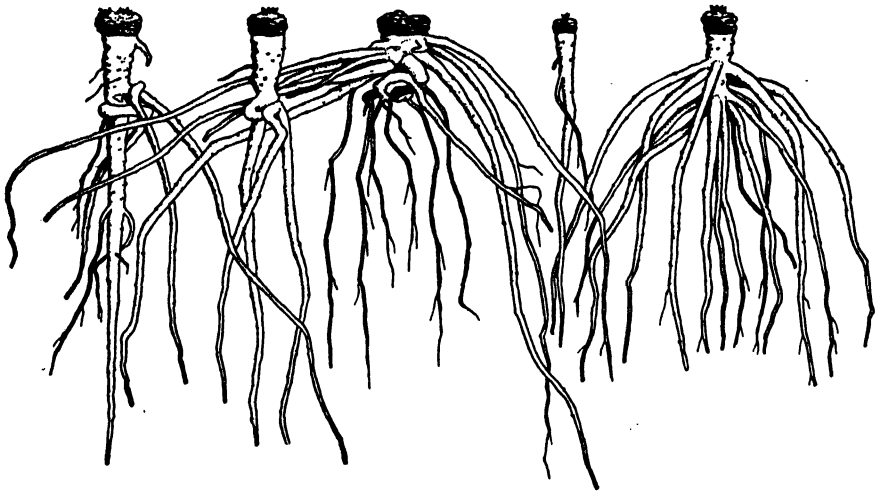


FIG. 3. Types of root systems in first-year tetraploids. From left to right weights were 64.5, 43.0, 81.0, 15.5, and 57.0 gm.

fibrous systems with numerous secondaries equalling or surpassing the tap root. Occasionally a tap root was not discernible. Analysis of the data on configuration of the root systems failed to reveal outstanding interfamilial differences, except that the crosses with the weightier roots generally had better developed secondaries.

Among the diploids the largest roots came from three-year-old achenes, the smallest from one-year-old achenes. Disbudding produced a slight increase in root size, but even the disbudded plants developed abnormally small roots. For some reason the derivatives of 1946 achenes were subnormal in all recorded growth characteristics such as leaf area, head production, and root size. They were inferior to random diploid plantings in previous years, to the parental series, and to the current year's plants grown from the same seed lot as their parents. The relative failure of this series could not be attributed to environmental factors in the garden plot. Rather it would appear to be due to a preponderance of genetically inferior individuals, despite the fact that some 74 plants were involved and the achenes were produced by open pollination of an equal number of parental plants, themselves grown from mixed achenes. Whatever the explanation may be, it is clear that the data from these plants have no comparative value. We would seem to have here an illustration of the fallacy of deriving conclusions from a small number of plants, especially when the species is as variable as *kok-saghyz*.

For comparison of  $4n$  and  $2n$  root weights in mixed populations reference may be made to Table IV. Here it will be seen that the average root weight of tetraploids grown in 1947 from two-year-old achenes was 35.9 gm., and that of diploids, 24.8 gm. These values compare with 31.3 gm. and 26.9 gm. for plantings of the previous year from similar seed lots, then one year old. It should be noted that the 1946 plantings were in 8-in. pots, those of 1947 in the garden plot, an environment more favorable to growth.

### Correlation of Morphological Characters

General relationships between rosette size, flowering, and root weight such as reported previously (3) were noted again in the current plantings. The data are presented in Table II for two groupings of  $4n$  crosses with slightly different combinations of growth characteristics, and for diploids grown from 1945 achenes. In each case there was positive correlation between number of leaves at maximum development, diameter of the rosette, and root weight, and an inverse relationship between first-year flowering and plant size.

TABLE II

RELATIONSHIPS BETWEEN FRESH ROOT WEIGHT, HEAD PRODUCTION, NUMBER OF LEAVES, AND SIZE OF THE ROSETTE IN FIRST-YEAR DIPLOIDS AND TETRAPLOIDS

Description	Root weight, gm.	No. of plants	Av. no. of heads	Av. no. of leaves	Av. diam. of rosette, in.
$4n$ (var. crosses)	To 19.9	14	19.4	46.5	11.9
	20-39.9	59	18.0	64.0	12.7
	40-59.9	86	7.0	77.3	14.2
	Over 60	50	0.7	98.3	15.8
$4n$ (var. crosses)	To 19.9	25	25.0	61.5	12.5
	20-39.9	75	16.4	76.2	14.8
	40-59.9	43	8.2	86.0	15.8
	Over 60	8	0	99.1	16.1
$2n$ (mixed 2-yr. achenes)	To 9.9	6	93.0	88.3	11.1
	10-19.9	20	93.9	89.5	11.5
	20-29.9	19	107.7	116.3	12.8
	Over 30	12	40.4	164.8	13.0

TABLE III

FRESH ROOT WEIGHT AND LEAF TYPE IN FIRST-YEAR PLANTS

Series	No. of plants	Av. root weight in grams of plants with leaf types as indicated			
		I and II	III	IV	V
$4n$ (var. crosses)	272	24.0	30.3	37.4	52.1
$4n$ (var. crosses)	261	24.5	30.8	46.3	53.6



TABLE IV

COMPARISON OF 1947 AND 1946 PLANTINGS FROM A MIXED SEED LOT

Description	Site	No. of plants	Av. no. of leaves	Av. diam. of rosette, in.	Av. no. of heads	Av. root weight, gm.
4n 1947, 2-yr. achenes	Field	39	63.0	12.8	17.9	35.9
1946, 1-yr. achenes	Pots	79	37.0	11.7	4.0	31.3
2n 1947, 2-yr. achenes	Field	57	116.6	12.6	88.4	24.8
1946, 1-yr. achenes	Pots	58	97.8	12.3	37.1	26.9

TABLE V

FIRST-YEAR FLOWERING IN TETRAPLOIDS AND DIPLOIDS

Type	Age of achenes	No. of plants	Plants flowering, %	Av. head production per plant
4n	2 years	39	48.5	17.9
2n	2 years	57	95	88.4
4n	1 year	565	28.5	9.3
2n	1 year	77	90	66.1

While the general relationships were as described above, individual plants varied considerably, some possessing diverse combinations of the different characters. This deviation was sometimes general for a whole family. For instance, in the reciprocals 2050  $\times$  2076 and 2076  $\times$  2050, leaf area was large (Fig. 1), considerably above normal for the various 4n populations, but root weight was definitely below average for the group (Table I). In contrast, the leaf area in 2073  $\times$  2050 and reciprocal was much smaller (Fig. 1), but the roots were bigger. Within each of these families, however, the majority of the plants conformed to the general pattern, the largest tending to have the biggest roots. It would appear from the data that rosette size and root weight are both heritable, and that while they are usually associated, some individuals and families possess different assortments of these characters.

The relationships between root weight and the seasonal behavior of the rosette were varied. In most cases the rosettes of large-rooted plants expanded faster and reached maximum size later than those of small-rooted sibs. In Fig. 4 it will be noted that the rosettes of the large plants in 2050  $\times$  2073 and reciprocal, with roots exceeding 60 gm., continued to expand until harvesting, whereas the small plants, with roots under 40 gm., went into a late-summer recession. In 1690  $\times$  2076, on the other hand, the rosettes of both large and small plants underwent reduction in late summer, but the onset of the rest period was delayed and the recession was of lesser extent in the large plants. Yet another condition was observed in 2050  $\times$  2076

and reciprocal. Here the large-rooted plants, as usual, had much greater leaf area than the small-rooted sibs, but maximum size was attained more or less simultaneously and subsequent reduction was of similar degree in both cases. Although much variation occurred, both in individuals and in families, there was a general tendency for the rosettes of the large-rooted plants to grow faster, attain maximum size later, and to recede less in the late summer, as illustrated by var. crosses, Fig. 4.

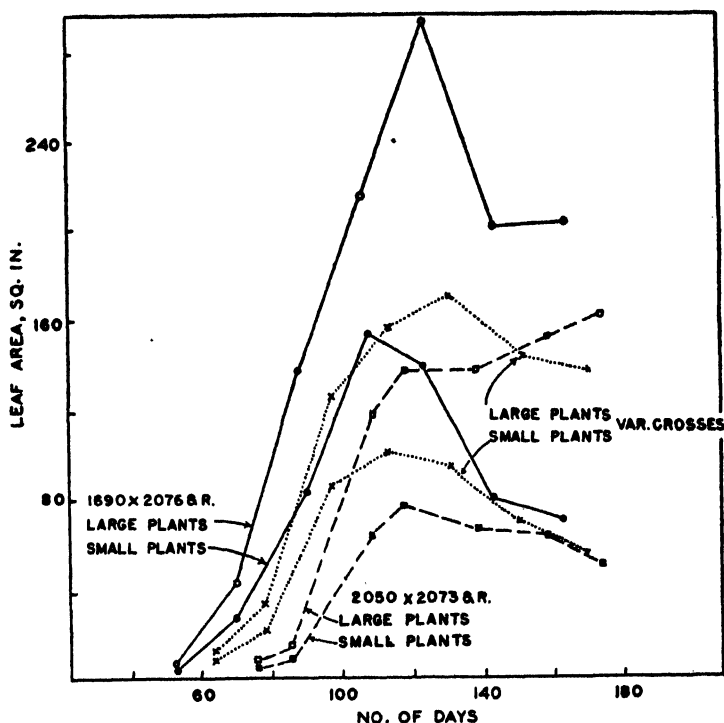


FIG. 4. Estimated leaf area of large plants with roots exceeding 60 gm. and of small plants possessing roots under 40 gm. in  $4n$  reciprocal crosses. Total, 167 plants.

Leaf shape and nature of margin were highly variable, but tended to cluster about modes specific for each  $4n$  family. As noted in a previous article (3), where the literature is reviewed, the observations of various authors indicate that leaf shape is determined by the interplay of ontogenetic, environmental, and genetic factors. As a rule  $4n$  progeny at maturity bore leaves resembling the parental types, but within each family some fluctuation occurred, with the largest plants tending to have the more deeply lobed leaves. Data on this relationship are given in Table III. Here the leaves are divided into five categories, with the lobing increasing from Types I to V as illustrated previously (3). The various  $4n$  crosses are divided into two groups, the first with leaves of Types III-IV predominating, the second with Types IV-V prevailing. In both groups the plants with the more deeply lobed leaves

(Types IV and V) tended to have larger roots. At the same time, of course, much individual variation occurred.

As referred to earlier, flowering has an inhibiting effect on root development. Scarth *et al.* (17) observed that disbudded diploids surpassed flowering plants, and Roberts and Struckmeyer (16) found flowering plants in a number of genera had smaller roots in relation to tops than nonflowering plants. Russian investigators have published differing reports on  $2n$  kok-saghyz (see Krotkov (9)). In both tetraploids and diploids described in the present paper plants with the biggest roots tended to flower least (Table II). Results from the 1947 plantings thus agree with those of 1946 (3) in demonstrating an inverse relationship between flowering and root development. The cumulative evidence indicates that two complexes of factors are involved in this relationship. First, the internal circumstances (hormone balance, etc.) that bring about flowering appear to have a deleterious effect on vegetative growth. Second, structural and food materials utilized in flower and seed production are not available for continued root development. That the first of these factors might be the more important is suggested by the results from the diploids grown from the year-old achenes. Here root weights were 12.7 gm. for flowering plants, 15.0 gm. for disbudded plants, and 25.0 gm. for nonflowering plants. However, it should be emphasized that the series of 74 plants was too small for the results to be conclusive. Evidence is required from other plantings before it is possible to assess the relative potency of the two factors.

It was hoped that the diploid plantings from one-, two-, and three-year-old achenes would provide information on the influence of age of achenes on plant development. Unfortunately, the obviously subnormal development of both vegetative and reproductive organs in the plants from year-old achenes prevented such comparison. In the 1946 plantings (3), it was noted that aging of the achenes for a second year induced a sharp reduction in flowering. This effect was not apparent in the 1947 plantings, both diploids and tetraploids from two-year-old achenes blooming well. The fact that the 1947 plants were transplanted in the garden plot, whereas those of 1946 were confined to 8-in. pots, although sunk outside, may have been a factor in maintaining a high level of flowering in the 1947 derivatives of two-year-old achenes. Cumulative evidence from various plantings during the past few years indicates that field conditions favor both more vigorous vegetative growth and greater flowering. Some data bearing on this question are presented in Table IV. Here it will be noted that both tetraploids and diploids produced bigger rosettes and more heads when removed from pots, but the response of the tetraploids to the more favorable environment surpassed that of the diploids. Root weight of the tetraploids showed a slight increase, that of the diploids a small decrease, possibly because of the upsurge in head production. Whatever the factors may be, the 1947 series of plot-grown plants did not exhibit the sharp recession in flowering among the derivatives of two-year-old achenes such as noted in the potted plants of 1946. However,

in the 1947 plants grown from three-year-old achenes, a decided falling off in flowering did take place (Table I). Germination in this group was reduced to one-third by the three years' aging of the achenes, but vegetative development of the survivors was not impaired. Actually the rosettes were leafier and the roots larger than in the other series, doubtless due in part to the reduced flowering. For the acquiring of satisfactory information on the effect of age of seed on plant growth it would seem necessary to repeat a number of specific crosses in successive years, in order that seed lots having a more or less similar range of genetic variability would be available for simultaneous planting.

The influence of seed size on plant growth has been investigated in many genera. The literature, reviewed by Oexemann (15) and Spurr (20) indicates that increased seed weight generally results in more vigorous initial growth but does not have a profound influence on size of plants at maturity. The writer came to a similar conclusion in the case of kok-saghyz (3). In the  $4n$  plantings described in the present article achene weight sometimes differed considerably between the cross and its reciprocal (Table I) so that there was here perhaps a better basis of comparison than when the achenes were selected from a mixed seed lot. In four families the bigger achenes produced larger plants, in two the reverse was true, and in the remaining two cases the results were inconclusive. On the whole it would appear that if achene weight did have a bearing on ultimate plant size, its effect was of minor importance as compared with that of other factors.

### Discussion

The general similarity in average root weight between  $4n$  reciprocal crosses, the differences between unrelated families, and the divergence between the progeny of large-rooted or small-rooted parents together indicate the existence of genetic factors in root development. Rosette size likewise appeared to be influenced by heredity. As a rule these characteristics were associated, plants with vigorous rosettes generally producing roots of corresponding size, but in some individuals and occasionally in whole families, different assortments of these characters occurred. Even in such cases, however, trends within the family were of the usual type.

The relationship between flowering and root development was of an inverse nature, the largest tetraploids generally bearing few or no inflorescences during the first year. Mashtakov (10), working with crowded diploid kok-saghyz, reported that the flowering plants produced the biggest roots, but other Russian authors disagreed (see Krotkov (9)). Scarth *et al.* (17) observed that disbudded  $2n$  plants surpassed those allowed to produce seed, and Roberts and Struckmeyer (16) found nonflowering plants in a number of genera to have consistently larger roots than flowering plants. Among our diploids there was a general tendency for the large-rooted plants to be less floriferous, and in the tetraploids the trend was more definite. The bulk of the evidence indicates that extensive flowering has an inhibiting effect on

root enlargement, or in other words the internal conditions associated with the reproductive phase retard vegetative growth.

An outstanding feature of tetraploids in the Toronto plantings has been their relatively sparse first-year flowering. In this regard our observations are at variance with those of Navashin *et al.* (13) who reported no difference between  $4n$  and  $2n$  kok-saghyz in proportion of plants flowering. Numerous authors (4, 6, 11, 12, 14, 18, 19, 21) have remarked on the slower maturing and later inception of blooming of tetraploids. Similar observations were recorded in the local plantings of kok-saghyz. The average interval between soaking of the seed and the first opening of capitula was 68 days in diploids grown from mixed two-year-old achenes and 82 days in the parallel series of tetraploids. As regards degree of floriferousness, the proportion of flowering plants and the average head production per plant were both decidedly lower in the tetraploids (Table V). It should be emphasized that this has been true for all local  $4n$  plantings, whether in pots or in the field (2 and 3).

The reduction in first-year flowering among tetraploids is highly significant because of its bearing on root growth. Since profuse flowering and seed production adversely affect root enlargement the tetraploids have an advantage over diploids in their sparse flowering. The biochemical basis of the relationships between vegetative and reproductive phases of growth has been the subject of considerable investigation. Some evidence has been adduced in support of the hypothesis that a specific floral hormone, which Čajlachjan (5) termed "florigen", is responsible for the onset of flowering, though attempts to extract it have met with failure and some investigators doubt its existence as a specific hormone. Galston (7) has raised the question of the possibility of a functional association of various growth regulating hormones in plants. He suggested that in such an association "florigen", assumed to promote flowering, was antagonistic to auxin favoring vegetative growth. Whatever the nature of the various substances having to do with growth may be, it would appear that the balance between these substances was more favorable to root development and less conducive to flowering in tetraploids than in diploids. As well as being affected by chromosome number it would also seem that this balance was influenced by heredity and a succession of environmental factors that probably begin with such circumstances as age of the seed and its pre-germination treatment. As regards auxin, Gustafson (8), Smith (19), and Avery and Pottorf (1) have reported lower concentration in tetraploids than in diploids, but more analytical work is required before general conclusions may be drawn. Obviously, such analysis should be accompanied by careful observations on morphology.

### Acknowledgments

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## STUDIES OF CANADIAN THELEPHORACEAE

### I. SOME NEW SPECIES OF *PENIOPHORA*<sup>1</sup>

BY H. S. JACKSON<sup>2</sup>

#### Abstract

In connection with a general taxonomic study of the resupinate Thelephoraceae of Canada, a number of forms have been encountered that appear to be undescribed. In this contribution nine such forms falling in the genus *Peniophora* are described and illustrated as probable new species.

#### Introduction

During the past 15 years the writer has had occasion to examine well over 7000 collections of resupinate Thelephoraceae from all parts of North America. Among such a large number of specimens it is perhaps to be expected that forms have been encountered that appear to be undescribed. Such forms have been accumulating for several years but available time for this study is necessarily limited and most of that time has so far been spent in attempting to become familiar with the old species, both European and American. It is only recently that the writer has developed sufficient confidence to venture to describe as new any of the odd forms that have been encountered.

Our knowledge of the Thelephoraceae of North America, particularly the resupinate forms, is still in a pioneer stage. Much remains to be done in straightening out the confusion that has resulted because of inadequate knowledge of the European species on the part of American mycologists. The writer is fully aware that to propose new species in this group involves considerable risk of merely adding to an already overburdened synonymy since there are many described species in the literature concerning which little is yet known. It has become evident, however, that it is impracticable to hope for complete knowledge of all the forms that have been recorded, many of which have been inadequately described. It is felt that progress will be better served by furnishing accurate illustrations and carefully prepared descriptions of forms that appear to be distinct after thorough study and the elimination of all the better known described species. Included in the present contribution are descriptions and illustrations of nine species of *Peniophora*, all of

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The writer is indebted to Miss Margaret H. Thomson for the preparation of all but one of the Latin diagnoses and to Miss Charlotte M. Dill for preparing the drawings. Miss E. Ruth Dearden has assisted in the final preparation of the descriptions and Dr. D. P. Rogers has furnished the description for *P. cymosa* and has read the manuscript.

which have been found in Ontario, which to the best of our knowledge appear to be undescribed.

The genus *Peniophora* is here interpreted in the generally accepted sense as including those species of corticioid resupinates having spores with essentially colorless, usually smooth walls, and that have cystidia in the hymenium. From a general survey of the species included in the genus, it is evident that to place the main emphasis on the character of presence of cystidia results in bringing together several groups of species that show little relationship between groups. In some species or groups it is evident that the real relationship is with species now included in *Corticium*, also a large collective genus, which as currently interpreted, includes most of the simpler resupinates of the group having no special inclusions in the hymenium other than gloeocystidia. In the case of other species it is evident that their relationship is with forms now included in one or another genus of resupinate Hydnaceae. The whole group of resupinate forms among Homobasidiomycetes is obviously in need of revision. Such revision, however, must be based on an intimate knowledge of a wide range of species and it is doubtful if anyone, certainly not the present writer, as yet possesses the broad and accurate knowledge of species that would be necessary before a complete revision could be proposed. Revision can perhaps best proceed progressively. When groups of species, which show obvious relationship, part of which may now be included in one genus and part in another, come to be thoroughly understood, they should be segregated.

Since very little generic revision has as yet been attempted, it seems best for the present and for the purpose of this contribution to include the species to be described in the genus *Peniophora* interpreted in the generally accepted sense.

### Description of Species

#### *Peniophora lauta* sp. nov. (Fig. 1)

Fructificatio alba vel glauca, tenuis, pruinosa, sub lente pilosa; basales hyphae paralleliter currentes, non nodoso-septatae; cystidia longa aciculataque  $80-120 \times 6.5-9 \mu$ , tunicis firmis levibus  $0.75-1.5 \mu$  crassis; basidia clavata  $20-30 \times 7-8 \mu$ , 4 sterigmatibus arcuatis  $5-8 \mu$  longis; basidiosporae late ellipsoideae vel subgloboseae  $6-8 \times 5-6 \mu$ , tunicis tenuibus levibusque.

Fructification a white or gray pruinose film spreading over surface of substratum in irregular small patches, pilose under a lens due to the long cystidia, basal hyphae more or less horizontal, few, wall firm, only slightly thickened, without clamps or incrustation; cystidia  $80-120 \times 6.5-9 \mu$  or more, arising directly from basal hyphae, long, acicular, walls smooth,  $0.75-1.5 \mu$  thick, tapering to a slender sometimes mucronate point; basidia arising terminally or laterally on upright branches from basal hyphae, often in clusters, clavate  $20-30 \times 7-8 \mu$  with four arcuate sterigmata  $5-8 \mu$  long; spores broadly ellipsoid to subglobose  $6-8 \times 5-6 \mu$  flattened on one side, often broadest below the middle, walls thin, smooth, not amyloid.



**Specimens examined:**

**Ontario:** On bark of *Thuja occidentalis*, Don Valley near Sunnybrook Park, Toronto, York Co., Sept. 17, 1942, 17581\* **type**; woods N.E. of Maple, York Co., Oct. 9, 1943, 18837; on bark of *Tsuga canadensis*, woods W. of Maple, July 15, 1945, 20120.

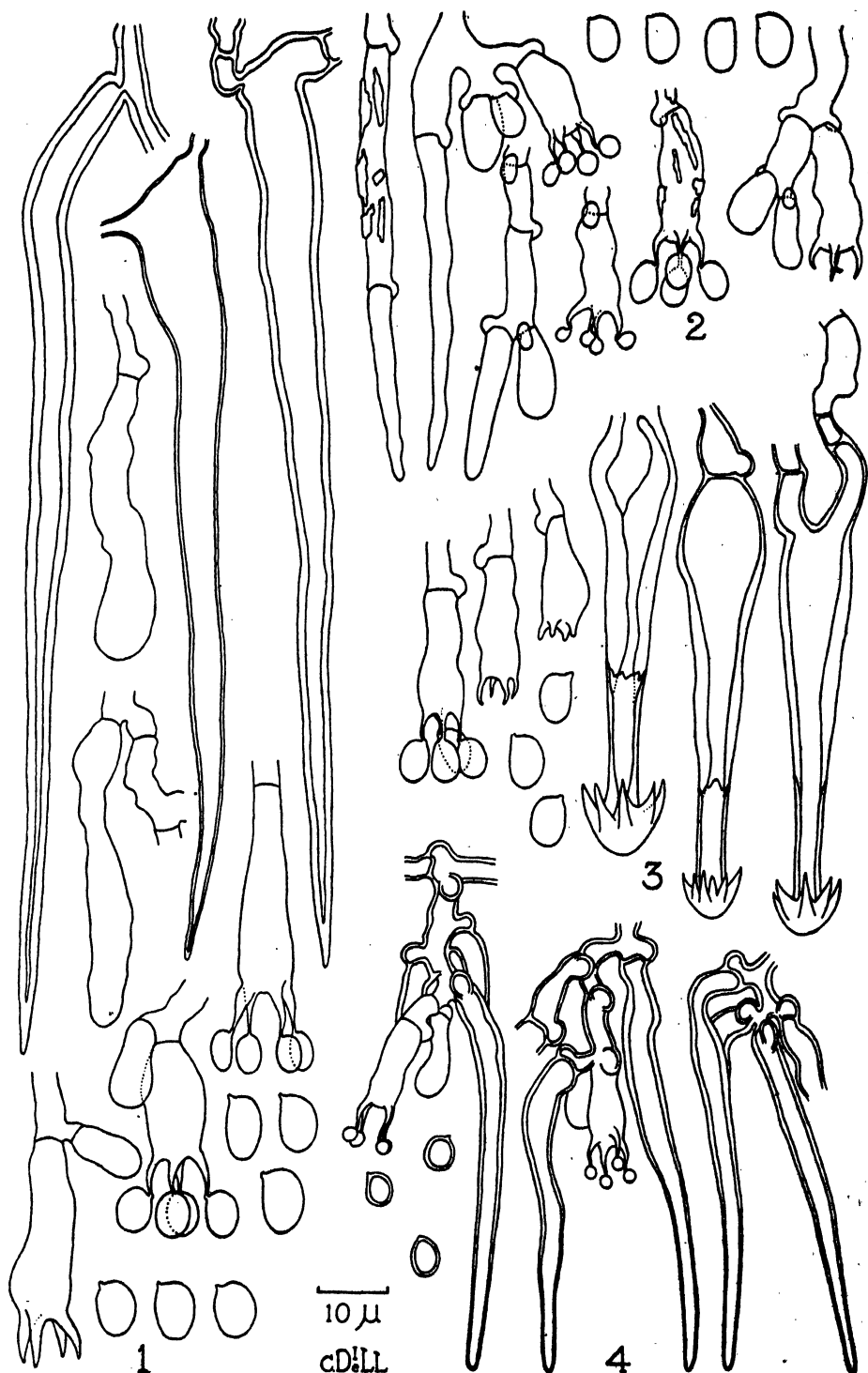
**Oregon:** On deciduous wood, W. of Corvallis, Benton Co., March 17, 1939, M. Doty 766, TRT.

*Peniophora lauta* would probably have been placed by Bourdot and Galzin (Hyménomycètes de France) in their subsection *C maculiformes* of the Gloeocystidiales. It seems probable that the species, as here described, will be found to include *P. populnea* Bourd. & Galz. The figure and description furnished by them strongly suggest that we are dealing with a form of the same species. *P. populnea* Bourd. & Galz. (1928) is, however, an untenable name, being antedated by the combination *P. populnea* (Peck) Burt (1926). Because of this situation and since we have not seen the type of the Bourdot & Galzin species, it has seemed best to describe our fungus as new rather than to provide a substitute name based on the type of *P. populnea* Bourd. & Galz.

The species is unique because of the subglobose spores and the very long slender cystidia with relatively thin but firm and smooth walls. As listed above, four collections have been available, three on coniferous bark and one on bark and wood of a deciduous tree. All have the essential characters of the species but differ in details of spore size, length and breadth of cystidia, etc. In the drawings for Fig. 1 the two outside cystidia and the upper group of spores are drawn from the type TRT 17581 while the center cystidium and the lower group of spores are drawn from TRT 18837. The third Ontario collection TRT 20120 differs in having the extreme tips of the cystidia mucronate and the spores essentially globoid 6-7  $\mu$  in diameter. The Oregon collection also has globoid spores, slightly smaller than in the Ontario collections. The spores are slightly different in each of the four collections but quite uniform in any one. Rather wide variability of the sort indicated is met with most frequently among species that lack clamps and may, perhaps, be correlated with homothallism.

It seems probable that homothallic forms in the Basidiomycetes have been derived from heterothallic ones by occasional haplonts changing in such a manner that the full life and nuclear cycle is carried out on a thallus originating from a single-nucleated spore. This may perhaps be brought about by mutation either in the haplont or the dikaryotic diplont. In any given heterothallic form in which such a tendency developed, a series of 'short

\* The herbarium numbers cited here and following, unless otherwise indicated, are those of the Cryptogamic Herbarium, University of Toronto (TRT). If no collector is indicated the collection was made by the writer.



cycled' mutants might originate successively or perhaps intermittently. Originating in this manner there might well be a tendency for variations inherent in the parent heterothallic species to be sorted out and fixed in the resulting series of homozygous lines. In the parent heterozygous form, because of repeated hybridization, such inherent variations would not be so noticeable when several collections are compared.

While it is not suggested that the absence of clamps is proof of homothallism, it is probable that many species lacking clamps are actually homothallic. The above explanation might seem to account for the variation indicated above for *P. lauta*. Other such variable forms lacking clamps include *Pellicularia pruinata* (Bres.) Rogers and *Pellicularia vaga* (Berk. & Curt.) Rogers, both of which show wide variation in spore size and shape. The group of species centering around *Peniophora cremea* (Bres.) Sacc. & Syd. and *P. affinis* Burt also show wide variation and are extremely difficult to evaluate.

### ***Peniophora perexigua* sp. nov. (Fig. 2)**

Fructificatio hypochnoidea, tenuis, pruinosa, alba; basales hyphae paucae 2.5–4  $\mu$ , nodoso-septatae tenuiter tunicatae, crystallis extensis incrustatae; cystidia gracilia acuminata 20–50  $\times$  2–3.5  $\mu$ , in apice e rectis hyphis emergentia; basidia cylindracea vel subclavata 12–17  $\times$  4.5–5  $\mu$ , 4 sterigmata gerentia; basidiosporae late ellipsoideae 6–7  $\times$  4–5  $\mu$ , tunicis tenuibus levibusque.

Fructification hypochnoid, forming thin, pruinose areas on the substratum, white, basal hyphae few, more or less horizontal, 2.5–4  $\mu$  in diameter with clamps at all septa, branching at right angles, wall thin, incrustated with elongated crystals; subhymenial hyphae upright also incrustated and with clamps; cystidia slender, acuminate, 20–50  $\mu \times$  2–3.5  $\mu$  often incrustated below, may be septate with clamps, arising terminally from upright hyphae, frequently with clusters of basidia at base; basidia often incrustated below, cylindrical or subclavate 12–17  $\mu \times$  4.5–5  $\mu$  with four slightly divergent sterigmata 3.5–4.5  $\mu$  long; basidiospores broadly ellipsoid 6–7  $\times$  4–5  $\mu$  somewhat flattened on one side with lateral apiculus, walls thin, smooth, non-amyloid.

Specimens examined:

**Ontario:** On bark of *Tsuga canadensis*, woods W. of Maple, York Co., Sept. 6, 1940, 16529; Oct. 5, 1940, 16531, 16532 type; Oct. 9, 1943, 18839; Sept. 8, 1945, 21803; Brewer Lake, Algonquin Park, R. F. Cain, Sept. 8, 1939, 14166; Nashville, York Co., R. F. Cain, Oct. 20, 1945, 21801; Oct. 22, 1945, 21800; on *Abies balsamea*, Bear Island, Lake Timagami, R. F. Cain, Aug. 1, 1939, 15114.

This species would appear to be more closely related to *P. pusilla* described below than to any previously described species. It differs, however, in the larger size of the basidia and in the shape and size of the spores. The habitat and gross appearance of the fructifications of the two species are similar, occurring most commonly on bark of hemlock.

**Peniophora hamata** sp. nov. (Fig. 3)

Fructificatio tenuis, alba, poroso-reticulata vel subpellicularis; subiculum paulum, hyphis non nodoso-septatis; cystidia obclavata  $50-75 \times 8-12 \mu$ , a convexis et in apice natis  $7-8 \times 8.5 \mu$  pileis qui in margine  $10-12$  decurvatis furcis  $3-4 \mu$  longis praediti sunt praepilata; basidia clavata cylindracea  $16-20 \times 6-7.5 \mu$ , 4 sterigmatibus  $3.5-4 \mu$  longis; basidiosporae late ellipsoideae  $5.5-7.5 \times 4-4.5 \mu$ , tunicis tenuibus levibusque.

Fructification white, thin, broadly effused with indeterminate margin, porous reticulate to subpelliculose, separable; subiculum scanty with clamped hyphae  $2.5-3 \mu$  broad, indistinct; cystidia obclavate,  $50-75 \mu$  long,  $8-12 \mu$  broad  $\frac{1}{2}$  distance from base,  $3.5-4.5 \mu$ ,  $\frac{1}{2}$  distance from apex, capitate with convex terminal caps  $7.5-8.5 \mu$  broad bearing  $10-12$  marginal decurved prongs  $3-4 \mu$  long; walls somewhat thickened especially in middle portion, this region swelling in potassium hydroxide, finally dissolving completely, terminal third and caps with thick walls not affected by potassium hydroxide; basidia clavate-cylindrical  $16-20 \times 6-7.5 \mu$  bearing four slender slightly arcuate sterigmata  $3.5-4 \mu$  long; basidiospores broadly ellipsoid  $5.5-7.5 \times 4-4.5 \mu$ , flattened on one side with prominent lateral apiculus, walls thin, smooth, non-amyloid.

Specimens examined:

**Ontario:** On coniferous wood, usually *Abies balsamea*. Bear Island, Lake Timagami, July 18, 1936, 10462; Aug. 8, 1936, 9866; Aug. 12, 1936, 9872; R. Biggs, July 17, 1936, 12488; July 21, 1936, 10818; Aug. 4, 1936, 9873; Aug. 6, 1936, 12483; Aug. 12, 1936, 9867, 9877; Aug. 19, 1936, 9848; R. F. Cain, Aug. 1, 1939, 15120, 15239; Gull Lake Portage, Lake Timagami, Aug. 10, 1936, 9946; Long Point, East Mainland, Lake Timagami, R. Biggs, Sept. 12, 1936, 9871 type; Gomphidius Bay, West Mainland, Lake Timagami, R. Biggs, Sept. 1, 1936, 9876; Costello Lake, Algonquin Park, R. F. Cain, Sept. 4, 1939, 14565, 14569 (on *Thuja occidentalis*); trail to Pine Tree Lake, Algonquin Park, Sept. 16, 1939, 14397.

The species described above is a member of the third xxx division of the section Tubuliferae of the Bourdot and Galzin classification. This division includes for the most part species with thick-walled cystidia, often with capillary lumen at the base, the walls of which are more or less affected by potassium hydroxide, often dissolving completely.

*P. hamata* is a unique member of this group because of the cystidial cap with downward projecting prongs. This cap is apparently of different composition and is not affected by potassium hydroxide while the lower part of the wall of the cystidium swells greatly in potassium hydroxide, finally dissolving more or less completely. The only other member of this section familiar to us, having an apical portion of the cystidium not affected by potassium hydroxide is *Peniophora inornata* described in this paper.

**Peniophora cymosa** Rogers & Jackson sp. nov. (Fig. 4)

Fructificatio tenuis, sicca minute pilosa, alutacea; hyphae plerumque crasse tunicatae, nodoso-septatae, contortae,  $3-4.5-7.5 \mu$  diam., altitudinibus variis vel basidium vel cystidium gignentes; cystidia subulata, acutata, ubique crasse tunicata,  $50-78 \times 4-6 \mu$ ; basidia cylindracea,  $10-12 \times 4.5-5.5 \mu$ , sterigmata quattuor gracilia, curvula, divergentia,  $3.5 \mu$  long. gerentia, tunica inferiori subincrassata persistentique; sporae globosae, crasse tunicatae, uniguttulatae,  $4.5-5 \mu$  diam.

Fructification thin, adnate, when fresh probably gelatinous-waxy, when dry buffy, discontinuous, minutely pilose; composed of distinct hyphal branches growing out perpendicular to the substratum and giving rise at brief intervals to cystidia and basidia—or better, repeatedly terminating in either a basidium or a cystidium, and repeatedly continued by acropetal growth of a lateral branch; hyphae short-celled with prominent clamps, mostly thick-walled, subarticulate,  $3-4.5-7.5\ \mu$ , the fertile branches sinuose and retaining fragments of the old discharged basidia still attached; cystidia slender-subulate,  $50-78 \times 4-6\ \mu$ , often somewhat curved, thick-walled throughout, acute, sometimes with a resinous droplet at the tip, arising at various levels from the fertile hyphae; basidia subcylindric, about  $10-12 \times 4.5-5.5\ \mu$ , arising at various levels, the lower wall thickened and persistent, bearing four slender, curved, divergent sterigmata  $3.5\ \mu$  long; spores subglobose,  $4-5\ \mu$  in diameter, slightly flattened beyond the apiculus, with appreciably thickened walls, smooth, guttulate, non-amyloid.

Specimens examined:

**Ontario:** On partly decayed coniferous wood, Bear Island, Lake Timagami, Aug. 19, 1936, R. Biggs, 10837, 10785.

**North Carolina:** Highlands, Aug. 17, 1933 (first foray), G. W. Martin, 1321, type, in TRT and IA.

*Peniophora cymosa* would perhaps fall in the section *Hyphales* of the Bourdot and Galzin classification. This section, however, as treated by them, includes a rather heterogeneous assortment of obviously unrelated forms none of which seem close to *P. cymosa*.

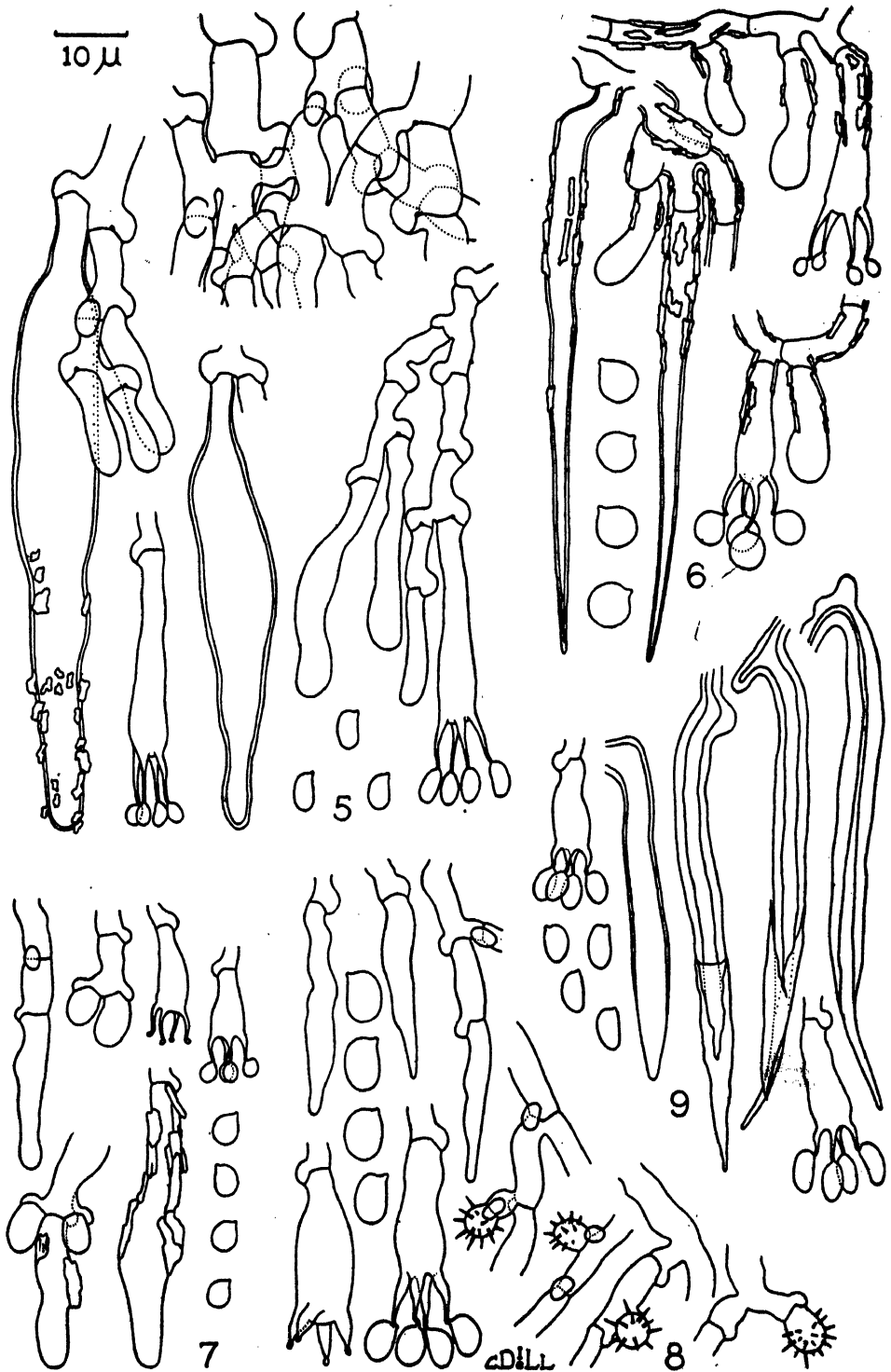
The unique features of the species here described are the thick-walled subglobose spores, and the uniformly thick-walled unincrusted cystidia, which are often more acutely pointed than brought out in the drawings (Fig. 4).

### *Peniophora probata* sp. nov. (Fig. 5)

Fructificatio late effusa, alba, dein isabellina,  $75-100\ \mu$  crassa; subiculum laxo intextis hyphis nodoso-septatis compositum; hyphae inferiores irregularibus e cellulis  $8.5\ \mu$  diam. brevibus; hyphae rectae et supra paralleles,  $2.5-3.5\ \mu$  diam., nodulosae; cystidia clavata vel ventricoso-clavata,  $55-70 \times 8-13\ \mu$ , tunicis inferioribus tenuibus, parum incrassatis, apicem versus curvulis; basidia gracilia clavata  $20-30 \times 4-4.5\ \mu$ , 4 sterigmatibus  $3.5-5\ \mu$  longis; basidiosporae ellipsoideae  $4.5-6 \times 2.5-3\ \mu$ , tunicis tenuibus levibusque.

Fructification white when young, becoming ochraceous to isabelline, broadly effused, separable,  $75-100\ \mu$  thick, hymenium becoming subceraceous; subiculum of loosely interwoven hyphae with clamps, upright and parallel in upper half  $2.5-3.5\ \mu$  in diameter, made up of short cells appearing nodulose due to prominent clamps, becoming broader below and connecting with a

FIG. 5. *Peniophora probata*; FIG. 6. *Peniophora ralla*; FIG. 7. *Peniophora pusilla*; FIG. 8. *Peniophora compta*; FIG. 9. *Peniophora inornata*, all showing cystidia, basidia, and spores. In Fig. 5 above, a group of wide subicular hyphae and in Fig. 8 at the right a group of aculeate bodies on the subicular hyphae. All figures drawn to a uniform scale with the aid of a camera lucida from potassium hydroxide-phloxine mounts and reproduced at an approximate magnification of  $1000\times$ .



looser subiculum made up of intertwined hyphae of irregular short cells varying in diameter to  $8.5\ \mu$ ; cystidia clavate or ventricose clavate,  $55\text{--}70 \times 8\text{--}13\ \mu$ , wall thin or becoming slightly thickened in upper half or third, rounded and often incrustated with crystals at apex; basidia slender clavate  $20\text{--}30 \times 4\text{--}4.5\ \mu$  extended slightly above level of developing basidia when mature, with four slender upright or slightly divergent sterigmata  $3.5\text{--}5\ \mu$  long; spores ellipsoid,  $4.5\text{--}6 \times 2.5\text{--}3\ \mu$ , flattened and appearing straight on one side with lateral apiculus, walls thin, smooth, non-amyloid.

**Specimens examined:**

**Ontario:** On decaying wood of *Pinus*, Bear Island, Lake Timagami, July 15, 1937, 13708; Aug. 5, 1937, 13709; July 22, 1935, 8905; Aug. 13, 1938, 13989; July 15, 1938, 13996; Aug. 12, 1943, 19090; Aug. 7, 1943, 18832, type; R. Biggs, Aug. 14, 1936, 12903; R. F. Cain, Sept. 7, 1935, 8663, July 19, 1939, 15288; on wood of *Tsuga canadensis*, woods W. of Maple, York Co., July 15, 1945, 20119.

According to the treatment of Bourdot & Galzin, this species would perhaps be included in the subsection *A submembranaceae* of their section *Gloeocystidiales*. It is however not a typical member of that group if such species as *P. argillacea* (Bres.) Sacc. & Syd., *P. medioburiensis* Burt, *P. clavigera* (Bres.) Bourd. & Galz., and *P. fusca* Burt represent the more typical forms. Our species may be separated from others in the genus by the combination of cystidial and spore characters taken together with the presence in the subiculum of broad hyphae made up of short, somewhat irregular cells as illustrated in the upper portion of Fig. 5. When crushed mounts are made in potassium hydroxide-phloxine, there is evident a considerable amount of oily material.

***Peniophora ralla* sp. nov. (Fig. 6)**

Fructificatio alba, delicata, pruinosa; hyphae subiculi paucae,  $3.5\text{--}4\ \mu$  diam., plerumque incrustatae, non nodoso-septatae; cystidia terminalia vel e basalibus hyphis emergentia, aciculata  $60\text{--}75 \times 4\text{--}5.5\ \mu$ , ad apicem acutatum attenuata, tunicis firmis leviterque incrassatis; basidia clavata vel subcylindracea,  $16\text{--}20 \times 4\text{--}5\ \mu$ , 4 sterigmata arcuata  $5\text{--}6.5\ \mu$  longa gerentia; basidiosporae globosae,  $5\text{--}6\ \mu$  diam., tunicis tenuibus levibusque.

Fructification white, delicate pruinose, interrupted; basal hyphae few, usually incrustated,  $3.5\text{--}4\ \mu$  in diameter, without clamps, wall thin but often appearing to be thickened because of incrustation; cystidia terminal or arising as upright branches from basal hyphae, acicular  $60\text{--}75 \times 4\text{--}5.5\ \mu$  at base, tapering to a fine point, wall firm, slightly thickened, appearing rugose toward base due to fine incrustation, with simple septum close to point of origin; basidia clavate to subcylindrical,  $16\text{--}20 \times 4\text{--}5\ \mu$  with four arcuate sterigmata  $5\text{--}6.5\ \mu$  long, often incrustated at base, arising directly as side branches of basal hyphae or in clusters; basidiospores globoid,  $5\text{--}6\ \mu$  in diameter, not appreciably depressed at one side, with prominent apiculus; walls thin, smooth, non-amyloid.

**Specimens examined:**

**Ontario:** On bark and wood of *Abies balsamea*, portage to Gull Lake, Lake Timagami, Aug. 10, 1936, 9770, 10789; R. Biggs, Aug. 10, 1936, 9771, **type**; Sept. 9, 1936, 10790.

*Peniophora ralla* might be compared to the forms included by Bourdot & Galzin in the second xx group of their section Tubuliferae as for example *P. clematidis* Bourd. & Galz. and *P. abietis* Bourd. & Galz. In both these species, however, the cystidia have very much thickened walls, rugose on the surface, and clamps are present in the hyphae. It is perhaps more closely related to *P. lauta*, described above, differing however in the shorter and more slender cystidia and in spore characters.

**Peniophora pusilla** sp. nov. (Fig. 7)

Fructificatio alba, divergens, pruinosa; subiculum infra laxis subparalleliter currentibus hyphis, 2.5–4  $\mu$  diam., nodoso-septatis, supra cymoso modo ramos emittentibus, floccosis crystallis crasse incrustatis; cystidia cylindracea vel clavata, in apice obtusa, 25–45  $\times$  4.5–6.5  $\mu$ , tenuiter tunicata; basidia cylindracea vel clavata, 6.5–10  $\times$  4–4.5  $\mu$ , 4 gracilia 3–4.5  $\mu$  longa sterigmata gerentia; basidiosporae subglobose 3.5–4.5  $\times$  3–3.5  $\mu$ , tunicis tenuibus levibusque.

Fructification white, forming a pruinose spreading growth on substratum usually not forming a continuous hymenium, margin indeterminate; subiculum formed of loose hyphae subhorizontal below, branching in a cymose manner above, lower hyphae with slightly thickened walls, 2.5–4  $\mu$  broad, occasionally inflated, clamps present, subhymenial hyphae thinner, heavily incrustated with flaky highly refractive crystals not readily soluble in potassium hydroxide that may extend to base of basidia and cystidia; cystidia cylindrical or clavate, arising from clusters of basidia, with thin walls, obtuse at apex, 25–45  $\times$  4.5–6.5  $\mu$  often with septum and clamp below center, emergent by about half their length; basidia cylindrical or clavate 6.5–10  $\times$  4–4.5  $\mu$  with four slender divergent sterigmata 3–4.5  $\mu$  long; basidiospores subglobose 3.5–4.5  $\times$  3–3.5  $\mu$ , depressed on one side, with minute obliquely placed apiculus, walls thin, smooth, non-amyloid.

**Specimens examined**, all on bark of *Tsuga canadensis*:

**Ontario:** Brewer Lake, Algonquin Park, R. F. Cain, Sept. 5, 1939, 14539.

**Quebec:** Burnet, M. Timonin, Oct. 1, 1935, OTB, F6785, **type**, TRT.

**New Hampshire:** Lac Lincoln, H. G. Eno, Sept. 27, 1939, BPI, FP 84884, TRT.

As previously noted under *P. perexigua*, this species is perhaps closely related. The basidia are however only about half the length of those in *P. perexigua* and the spores are much smaller and of different shape. Both species have a similar type of incrustation on the hyphae, which may be correlated with the similar substratum. No difficulty is encountered in separating the two from the microscopic characters.



***Peniophora compta* sp. nov. (Fig. 8)**

Fructificatio tenuis, divergens, alba vel crenea, subpellicularis; subiculum ex hyphis laxè intricatis, 3.5–5.5  $\mu$  diam., nodoso-septatis, tunicis firmis partimque leviter incrassatis praeditis, ad basim vel ad apicem structuræ quæ globosae vel subglobosae cellulae 3.5–5.5  $\mu$  diam. sunt hic et illic ferentibus compositum, tunicis tenuibus parce distributis aculeatis spinis 0.75–1.5  $\mu$  longis praeditis; cystidia gracilia, acuminata 25–45  $\times$  2.5–4.5  $\mu$ , tunicis tenuibus interdum septatis; basidia clavata vel subcylindracea 15–25  $\times$  6.5–8.5  $\mu$ , 4 sterigmata 4.5  $\mu$  longa gerentia; basidiosporae late ellipsoideae 5.5–8  $\times$  3.5–5  $\mu$ , tunicis tenuibus levibusque.

Fructification thin spreading, whitish or cream, subpelliculose, margin indeterminate, separable and friable when dry; hyphae of subiculum loosely arranged, with regular clamps, walls firm, in part slightly thickened, of varying diameter, 3.5–5.5  $\mu$ , bearing occasional aculeate bodies consisting of a globose or subglobose cell 3.5–5.5  $\mu$  in diameter, sessile or terminal on short branch, always subtended by a clamp, wall thin with sparsely distributed aculeate spines 0.75–1.5  $\mu$  long; cystidia (or cystidioles) slender, acuminate, 25–45  $\times$  2.5–4.5  $\mu$ , somewhat flexuous, arising in the hymenium or subhymenium and extending beyond basidia for  $\frac{1}{3}$  –  $\frac{1}{2}$  length, with septum and clamp at base or occasionally also at  $\frac{1}{3}$  –  $\frac{1}{2}$  from base to apex, walls thin not incrusted; basidia clavate or subcylindrical 15–25  $\times$  6.5–8.5  $\mu$  bearing four straight or slightly divergent sterigmata 4.5  $\mu$  long; basidiospores broadly ellipsoid, 5.5–8  $\times$  3.5–5  $\mu$  slightly flattened and appearing nearly straight on one side, with lateral apiculus, walls thin, smooth, non-amyloid.

**Specimens examined:**

**Ontario:** On decayed coniferous wood, frequently *Pinus Strobus*. Bear Island, Lake Timagami, July 21, 1939, 15019 type; Aug. 18, 1944, 19742, 20030; Paradis' Bay, Lake Timagami, Aug. 22, 1935, R. Biggs, 8746; Aug. 26, 1936, 16675; woods S. of Aurora, York Co., Oct. 16, 1938, 13717, 13718.

The cystidia in this species are of the type that might be referred to as cystidioles and the species could perhaps as well be included in *Corticium* as in *Peniophora*.

In morphological details of cystidia and spores, as indicated by the drawings (Fig. 8), this species might appear to be close to *P. perexigua* (Fig. 2), but in gross appearance the two are quite different. In *P. compta* the cystidia arise in the hymenium perhaps as modified basidia, while in *P. perexigua* they arise from the subiculum.

The unique feature of *P. compta* is the presence of the characteristic aculeate bodies on the hyphae of the subiculum. So far as we are aware no structure of this sort has been previously described in any thelephoraceous resupinate. These structures are certainly not of the same nature as the caliciform bodies that are described for *P. caliciferum* by Litschauer (Oesterr. Botan. Z. 77 : 126. Fig. 4. 1928) and that also occur in other forms of the *P. tenuis* (Pat.) Massee complex. There is no suggestion that they are disseminated and they do not appear to be chlamydospores. Their function is obscure.

**Peniophora inornata** Jackson & Rogers sp. nov. (Fig. 9)

Fructificatio tenuis, alba, pruinoso, sub lente hispida; hyphae subculi plurimum paralleliter currentes, 3–3.5  $\mu$  diam., tunicis interdum leviter incrassatis, nodoso-septatae; cystidia infra cylindracea, ad acutatum apicem attenuata, pileo acuto tecta, 50–75  $\times$  4–5.5–7  $\mu$ , tunicis angusto lumine, infra crassis, supra tenuibus, in KOH praeter pileum acutum dissolubilibus; basidia cylindracea, 10–16.5  $\times$  4.5–6  $\mu$ , 4 sterigmata gerentia; basidiosporae ellipsoideae 4.5–5.5–6  $\times$  2.5–3.5  $\mu$ ; tunicis tenuibus levibusque.

Fructification a thin, white, delicate pruinose film separating on drying to form a fine reticulum, hispid under a lens from the numerous cystidia; hyphae of subiculum somewhat obscure, mostly horizontal, 3–3.5  $\mu$  in diameter, in part with slightly thickened walls and with clamps at septa; cystidia arising from upright or from basal hyphae, 50–75  $\times$  4–5.5–7  $\mu$ , cylindrical below, tapering above to an acute apex that is sheathed by an acutely pointed cap; wall thick below with narrow lumen, thin above, soluble in potassium hydroxide, except for the cap of different composition; basidia cylindrical 10–16.5  $\times$  4.5–6  $\mu$  with four sterigmata; basidiospores ellipsoid, 4.5–5.5–6  $\times$  2.5–3.5  $\mu$ , somewhat flattened and appearing straight on one side with lateral apiculus, walls thin, smooth, non-amyloid.

Specimens examined:

**Ontario:** On coniferous wood, usually *Abies balsamea* or *Pinus Strobus*. Bear Island, Lake Timagami, Aug. 8, 1944, 20011; Aug. 16, 1945, 21804; Aug. 21, 1944, 20032; Aug. 17, 1943, 19091; Paradis' Bay, Lake Timagami, R. Biggs, Aug. 17, 1936, 10838, 10839; Timagami Island, Lake Timagami, R. F. Cain, July 21, 1937, 13720; portage to Spawning Lake from Lake Timagami, Aug. 11, 1939, 15004 type; Opeongo Lake, Algonquin Park, R. F. Cain, Sept. 18, 1939, 14899.

**Oregon:** On *Pinus contorta*, Sutton Lake, Nov. 26, 1937, A.M. and D. P. Rogers, 424 also TRT; on *Pseudotsuga taxifolia*, Chetco River, above Brookings, July 12, 1939, A.M. and D. P. Rogers 764 also TRT.

The species here described is another member of the third xxx division of Bourdot & Galzin's section Tubuliferae. It is distinguished from the other members of the group primarily because of the acute caps over the tips of the cystidia. This cystidial cap, as in *P. hamata*, is of different composition from the thickened wall of the cystidium below. At first glance the thickened cap appears to blend insensibly into the wall below but when treated with potassium hydroxide the lower wall dissolves completely while the acutely pointed caps remain unaffected. These caps appear to develop independently of the main wall of the cystidium as the latter matures. Young cystidia show no signs of a cap and occasionally a nearly mature cystidium is found without a cap, in which case the upper part of the cystidium wall is shown to be quite thin and developed to an acute apex as shown in the cystidium farthest to the left in Fig. 9. It seems possible that these caps are formed as a secretion from the wall.

This species was recognized independently as new by Dr. D. P. Rogers from collections made in Oregon and I am indebted to him for the specimens from that state recorded above.

## A HOMOTHALLIC, SHORT-CYCLE STRAIN OF *UROMYCES* *POLYGONI* (PERS.) FUECKEL<sup>1</sup>

BY A. M. BROWN<sup>2</sup>

### Abstract

A strain of *Uromyces Polygoni* (Pers.) Fuckel collected on *Polygonum aviculare* L. at Winnipeg was found to be homothallic. Attempts to infect *P. erectum* L. and *P. neglectum* Besser with sporidia, aeciospores, and urediospores of this rust were unsuccessful. Of the sporidial infections on *P. aviculare*, whether originating from a single sporidium or from two or more sporidia, most produced aecia only, but some produced uredia only, and a few produced aecia and uredia in association. Pycnia were not visible in any of the sporidial infections when examined with a hand lens but, in microtome sections cut from infections that had developed aecia, a few pycnia containing pycniospores were observed. No flexuous hyphae and periphyses were seen in these pycnia.

### Introduction

*Uromyces Polygoni* (Pers.) Fuckel occurs yearly in Western Canada and commonly inhabits species of *Polygonum* (1, 2, 4). At Winnipeg, Man., in the summer of 1942, and again in 1943, this rust was observed on *Polygonum aviculare* L. but not on *P. erectum* L. or *P. neglectum* Besser, although the three species grew in close proximity to each other. These observations suggested that a strain of *U. Polygoni* having a restricted host range was present in the neighborhood of Winnipeg. To ascertain the validity of this assumption, infection experiments were undertaken. These had as a further objective the determination of the sexual behavior of this rust.

### Materials and Methods

Telial material of *U. Polygoni* was collected from *P. aviculare* in October, 1943. It was kept in paper bags in the laboratory at ordinary room temperature during the ensuing winter months. In April, 1944, teliospore germination was induced by subjecting selected small portions of the telial material to alternate wetting and drying. During the months of May and June, seedling plants of *P. aviculare*, *P. erectum*, and *P. neglectum* were enclosed in moist chambers and germinating teliospores were suspended over them, in a manner similar to that described by Craigie (3).

### Experimental Results

From 12 to 14 days after the plants had been exposed to sporidial showers, aecia or uredia, and in some cases both aecia and uredia, developed on *P. aviculare*, but no rust developed on *P. erectum* or *P. neglectum*.

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Contribution No. 924 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa.

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Although many isolated and compound infections occurred on *P. aviculare*, which proved to be a congenial host, in none of them could pycnia or pycnial nectar be seen, even with the aid of a hand lens. In microtome sections cut from monosporidial infections that had developed aecia, a few pycnia were observed. Pycniospores were present at the ostioles of a few of these pycnia but flexuous hyphae and periphyses were absent. Sporidial infections that had produced uredia only, or aecia and uredia in association, were not examined in microtome sections to determine the presence or absence of pycnia.

To obviate the chance transfer of pycniospores by insects from one infection to another, all of the infected plants were enclosed in wire-screen cages.

Altogether 110 well isolated, and apparently monosporidial infections, were observed and, of these, 96 produced aecia spontaneously when they were from 12 to 14 days old. Of the others, nine infections produced uredia but no aecia, and five produced both uredia and aecia.

As insects had been excluded from the infected plants and as no attempt was made to transfer pycniospores from one infection to another, the development of aecia and uredia in these monosporidial infections apparently resulted from nuclei contained within the individual thalli. Even if the few pycniospores observed in some infections had been fortuitously transferred from one infection to another, it is doubtful if, in the absence of flexuous (receptive) hyphae, their intervention would have been effective.

In addition to the infections just mentioned, 20 compound infections, that is, infections originating from two or more sporidia, were selected for observation. Of these infections, 14 produced aecia simultaneously in both components, two produced uredia in both components, and four produced aecia in one component and uredia in the other.

Examination of microtome sections cut from monosporidial infections indicated that the binucleate phase appeared first in the aecial primordia. Some incipient aeciospores developing from primordial aecial cells were observed to be uninucleate, although most were binucleate.

As previously mentioned, this rust appeared under natural conditions to be limited to *P. aviculare*. The problem of its host range was further investigated by sowing aeciospores and urediospores separately on well established, rust-free plants of *P. aviculare*, *P. erectum*, and *P. neglectum*. The inoculations were repeated a number of times with identical results: rust developed only on *P. aviculare*.

### Conclusions

The evidence just presented shows that the strain of *U. Polygoni* investigated has a restricted host range. Its sporidia, aeciospores, and urediospores, although repeatedly sown on *P. aviculare*, *P. erectum*, and *P. neglectum*, caused infection of the first-named species only.

Isolated monosporidial infections of *U. Polygoni* produced aecia spontaneously without the intervention of pycniospores. Some infections produced

uredia instead of aecia, and a few produced both aecia and uredia simultaneously and in association, presumably on the same mycelium. Evidently the rust mycelium present in each of these infections contained within itself the nuclear elements necessary to initiate the conjugate nuclear condition. On the basis of this evidence it is concluded that this strain of *U. Polygoni* is homothallic.

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# STUDIES OF CANADIAN THELEPHORACEAE

## II. SOME NEW SPECIES OF *CORTICIUM*<sup>1</sup>

By H. S. JACKSON<sup>2</sup>

### Abstract

In the following pages nine species of *Corticium*, found in Ontario and presumed to be new to science, are described and illustrated. These were encountered in connection with a general taxonomic study of resupinate Thelephoraceae. In addition, a redescription and discussion of a species recently published from Europe, also found to be common in Ontario, is included.

### Introduction

The general remarks included in the introduction of the first number of this series (See Footnote 1) will for the most part be applicable to the present contribution as well, except where they refer specifically to the genus *Peniophora*.

As was true of the first, this second paper is the result of a general study of the resupinate Thelephoraceae of North America, with special reference to those found in Canada, during which a large number of specimens have been examined. In the pages that follow, nine species falling in the genus *Corticium*, which to the best of our knowledge are new to science, are described, illustrated, and discussed. In addition a redescription of *Corticium sulfureo-isabellinum* Litschauer, which was described in a paper by Pilát (4) published in 1940 in Europe and which has been found to be common in Ontario, is included with detailed discussion.

The genus *Corticium* as usually delimited includes a large assemblage of simple resupinate species having no special inclusions other than gloeocystidia or cystidioles, and usually having colorless spores. Recent European students of the group have for the most part segregated those species possessing gloeocystidia in the genus *Gloeocystidium* H. & L. American students, on the other hand, have included the gloeocystidiate species in *Corticium* on the grounds that to segregate such forms on the basis of a single character merely results in another collective genus composed of species or groups of species that are unrelated. In any case *Gloeocystidium* H. & L. is an untenable name, being antedated by *Gloeocystidium* Karst. *Gloeocystidiellum* Donk, with *Corticium*

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The writer is indebted to Miss Margaret H. Thomson for the preparation of the Latin diagnoses, to Miss Charlotte M. Dill for the preparation of the drawings, and to Miss E. Ruth Dearden for assistance in the preparation of the technical descriptions. Dr. D. P. Rogers has been kind enough to read the manuscript.

*porosum* B. & C. as its type, is an available generic name for the gloeocystidiate forms. This generic name, because of its type, should perhaps be used, if at all, only in a restricted sense to include certain of the species having amyloid spore walls.

Since *Corticium*, like *Peniophora*, is obviously a collective genus, including a heterogeneous assemblage of unrelated groups of species it seems best for the present to retain the gloeocystidiate forms therein, until the relationships of such forms are better understood. In the present contribution two such species are included.

As was noted in the first paper of this series the resupinate Homobasidiomycetes are greatly in need of revision. The genus *Corticium* is no exception. Species or groups of species now included in this assemblage include the basic members of several natural evolutionary lines, some of which will undoubtedly be found to include species now in other genera of the Thelephoraceae, as well as species now included in various genera of the resupinate Hydnaceae and in the genera *Merulius* and *Poria* of the older concept of the Polyporaceae.

For the future one can envision a classification of the simpler Homobasidiomycetes based upon that first proposed by Patouillard (3), which has been elaborated by Bourdot and Galzin (1) and more recently by Donk (2). In this classification, the old family name Thelephoraceae will probably disappear and its place be taken by a series of families grouped under the ordinal name Aphyllophorales, some of the families perhaps divided into subfamilies and tribes. The basis of one or more of these families, subfamilies or tribes will emerge from a regrouping of species now or formerly in *Corticium* combined with species from other genera or families.

Recent segregates, illustrative of this trend in classification and having their basic species formerly in *Corticium*, which appear to be acceptable, include *Ceratobasidium* Rogers (5) and the related genus *Pellicularia* Cooke (= *Botryobasidium* Donk) (Rogers (6)), and *Trechispora* Karsten as revised and reviewed by Rogers (7). In *Pellicularia*, species formerly in *Corticium* and *Peniophora* are included. In *Trechispora* species formerly in *Corticium* are combined with others formerly in the genus *Grandinia* of the Hydnaceae and in *Poria* of the Polyporaceae. The closely related genus *Sistotrema* also formerly in the Hydnaceae includes properly only one or two pileate species. In all the segregates mentioned, the basic character considered of phylogenetic significance centers in the type of basidium.

Other groups of related species in *Corticium* can be more or less clearly recognized. Most of the species in the section *Humicola* of the Bourdot & Galzin classification form a natural group when combined with such species as *Sistotrema varicolor* Bourd. & Galz. and certain of the rough spored species of *Poria* such as *P. candidissima* (Schw.) Cooke. For this group the generic name *Phlebiella* Karsten, with *Corticium sulphureum* (Pers. ex Fries) Fries (= *Hypochnus fumosus* sensu Burt) as the type, is apparently available (Rogers (7, p. 79)). Some or all of the species of *Phlebia* when compared

with certain ceraceous species now in *Corticium* and *Peniophora* will probably be found to form a natural assemblage. Gloeocystidiate species such as *Corticium* (*Gloeocystidium*) *pallidum* Bres. when combined with the more characteristic members of Bourdot and Galzin's section *Gloeocystidiales* of *Peniophora* form a natural group. Certain species of *Corticium* will undoubtedly be found to have their nearest relatives among colorless-spored species of *Merulius*. Other examples might be cited but the cases mentioned are sufficient for the present discussion.

As such groups come to be more clearly understood and their limits established they should be segregated. As segregation proceeds the relationships of the remaining species should become more clear. Fortunately, students of the group are so far proceeding slowly and cautiously in segregating such groups. A major problem, which must be basic to any sound revision, is to determine what characters are of phylogenetic significance and reflect real relationships.

While the writer is in full sympathy with the necessity for revision, it seems undesirable at present to describe species and assign them to available genera until such genera are covered by a monographic or at least a synoptical treatment. For this reason the species described below are all assigned to *Corticium*. As revision proceeds, a proper place will be found for them in other genera.

### Description of Species

#### *Corticium delectabile* sp. nov. (Fig. 1)

Fructificatio effusa, crenea vel citrula, subceracea, 140–150  $\mu$  crassa; basales hyphae subiculi paucae, fere rectae, non nodoso-septatae, 3.5–4  $\mu$  diam., tunicis tenuibus, hyalinis; gloeocystidia nulla; basidia magna, 50–80  $\times$  7–9  $\mu$ , cylindracea vel subclavata, flexuosa, interdum subventricosa, 3 vel 4, raro 2 vel 5 arcuata subulataque sterigmata gerentia; basidiosporae subglobosae vel lacrimiformes 7–9  $\times$  6–8  $\mu$ , tunicis tenuibus, hyalinis, levibus.

Fructification effused, cream to yellow, subceraceous, adnate, 140–150  $\mu$  thick, margin indeterminate, horizontal basal subicular hyphae few, hyphae for the most part upright, without clamps, 3.5–4  $\mu$  in diameter, branching often pseudo-dichotomously, wall thin, colorless; gloeocystidia none; basidia cylindrical to subclavate, flexuous and occasionally subventricose, 50–80  $\times$  7–9  $\mu$ , bearing three or four, rarely two or five arcuate, subulate sterigmata 7–8.5  $\mu$  long; basidiospores in side view subglobose, laterally depressed with prominent apiculus, in face view subglobose to lacrimiform, 7–9  $\times$  6–8  $\mu$ , walls thin, hyaline, smooth, non-amyloid.

Specimens examined:

**Ontario:** On partly decayed, decorticated deciduous wood, woods W. of Maple, York Co., R. F. Cain, Oct. 1, 1938, 13684\* type; on humus, woods S. of Aurora, York Co., Oct. 10, 1937, 16722; on ground in woods, Don Valley, Toronto, York Co., R. F. Cain, Sept. 22, 1934, 8672; woods W. of Maple, Sept. 22, 1940, 16432.

\* The herbarium numbers cited here and following, unless otherwise indicated, are those of the Cryptogamic Herbarium of the University of Toronto (TRT). If no collector is named the collection was made by the writer.



The relationship of this species is not clear. It has some resemblance to *C. Macounii* Burt but differs in essential respects. In the latter species clamps occur at the septa, gloeocystidia and hyphal paraphyses are present, and the basidia are typically two-spored.

Gloeocystidia seem to be absent in *C. delectabile* though young basidia might easily be mistaken for them. The basidia are long, narrow, and flexuous, somewhat resembling those in some species of *Aleurodiscus*, but the species seems more properly referred to *Corticium*. The unique features are the long basidia and the characteristic spores.

### ***Corticium electum* sp. nov. (Fig. 2)**

Fructificatio tenuis, 50–75  $\mu$ , late effusa, alba vel eburneo-citrula, viva cereo-ceracea, sicca pruinosa vel farinosa, deinde continua, membranacea, levis vel minute rimosa; subiculum ex hyphis laxe intricatis, saepe ramosis nodulosisque 1.5–3  $\mu$  diam. nodoso-septatis compositum; gloeocystidia nulla; basidia gracilia cylindracea, 20–30  $\times$  2.5–3.5  $\mu$ , infra subventricosa, maturitate supra hymenium emergentia, 4 gracilia leviter divergentia sterigmata 4.5  $\mu$  longa ferentia; basidiosporae late reniformes 3.5–4  $\times$  2–2.5  $\mu$ , tunicis tenuibus, subtiliter echinulatis, in solutione KOH levibus, valde amyloideis.

Fructification 50–75  $\mu$  thick, becoming widely effused on decorticated wood, white or ivory yellow to pinkish buff, when fresh waxy ceraceous, when dry pruinose to farinose, becoming continuous, membranous, smooth or minutely rimose; subiculum of more or less upright loosely interwoven, frequently branched nodulose hyphae, with clamps, 1.5–2  $\mu$  in diam., becoming collapsed and indistinct; typical gloeocystidia absent; basidia slender cylindrical, 20–30  $\times$  2.5–3.5  $\mu$ , somewhat ventricose and broader below, extruded beyond the level of the hymenium at maturity, bearing four slender straight or slightly divergent sterigmata 4.5  $\mu$  long; basidiospores broadly reniform 3.5–4  $\times$  2–2.5  $\mu$  with inconspicuous lateral apiculus, walls thin, delicately and closely echinulate, strongly amyloid in Melzer's solution, becoming smooth in potassium hydroxide.

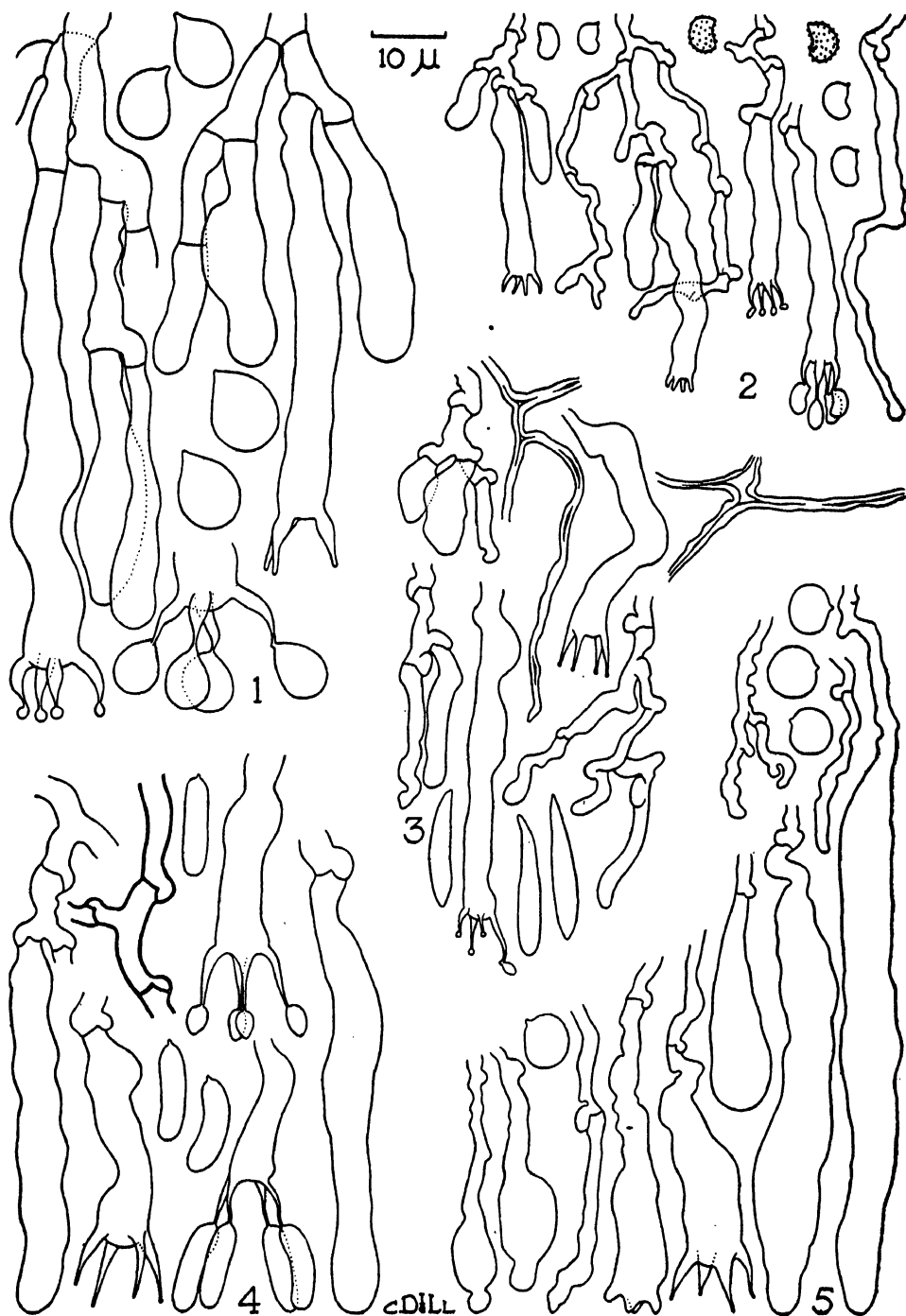
### **Specimens examined:**

**Ontario:** On decorticated wood of *Pinus* sp. Bear Island, Lake Timagami, Aug. 23, 1939, 14982 type; July 21, 1939, 14981; Aug. 1938, 17698; Aug. 17, 1943, 19082, 19085, 19094, 19100; on decorticated wood of *Thuja occidentalis*, Oakland swamp, Brant Co., R.F. Cain, June 12, 1939, 16569; Oct. 21, 1946, 21394; Hatchley, Brant Co., Oct. 11, 1943, 18791; highway between milepost 23 and 24 Algonquin Park, Sept. 17, 1939, 21860.

This species is apparently quite common on decorticated coniferous wood in Ontario but has not been encountered elsewhere. Branched tortuous hyphae are sometimes included in the hymenium as hyphal-like paraphyses.

FIG. 1. *Corticium delectabile*, basidia and spores. FIG. 2. *Corticium electum*, basidia, paraphyses, and spores. FIG. 3. *Corticium praestans*, basidia, spores, and subicular hyphae. FIG. 4. *Corticium definitum*, basidia, spores, gloeocystidia, and basal hyphae. FIG. 5. *Corticium sulfureo-isabellinum* Litsch., basidia, spores, gloeocystidia, and paraphyses.

All figures were drawn with the aid of a camera lucida at a uniform scale and reproduced at a magnification of approximately 1000 $\times$ .



Species of *Corticium* having the spore wall strongly amyloid and roughened are not found outside the group of species commonly included by European students of the group in the genus *Gloeocystidium*. It is possible that the species here described may be related to *Gloeocystidium furfuraceum* (Bres.) Höhn & Litsch., which is also common in Ontario. The present species differs in spore size and shape and in the absence of typical gloeocystidia. What appear to be immature basidia often occur having somewhat resinous contents, but these do not develop to structures that would properly be referred to as gloeocystidia.

The spore walls appear definitely roughened when mounted in water or in Melzer's solution but this roughening disappears when potassium hydroxide is included in the mounting medium. A similar reaction has been noted in other cases particularly in the genus *Aleurodiscus*. The now almost universal use of potassium hydroxide - phloxine as a mounting medium by students of this and related groups may result, in such cases, in spore walls being described as smooth unless some other medium is used as a check. The writer has adopted the practice when dealing with an unknown form of always making a crushed mount in Melzer's solution as a test for amyloidity and this procedure answers for observation of obscure roughening of the spore wall as well.

### ***Corticium praestans* sp. nov. (Fig. 3)**

Fructificatio alba vel crenea, divergens vel e locis orbicularibus coalescentibus emergens, firma, coriacea, tenuis 40–50  $\mu$ ; subiculum, plerumque e dense intricatis fibrosis hyphis 0.8–2.5  $\mu$  diam. crasse tunicatis, luminibus capillaribus aut absentibus, sed etiam partim ex hyphis tenuiter tunicatis, intricatis, nodoso-septatis, tandem basidia gerentibus compositum; gloeocystidia absentia; basidia 25–30  $\times$  5–6  $\mu$ , infra ventricosa vel subventricosa, supra longa cylindracea flexuosa, 4 rectis 5–5.5  $\mu$  longis sterigmatibus; basidiosporae subclavatae, subarcuatae, 12–14  $\times$  2.5–3  $\mu$ ; tunicis tenuibus, hyalinis, levibus.

Fructification white to cream, spreading, often from coalescing orbicular patches, margin determinate, thin 40–50  $\mu$ ; in texture firm, felty; subiculum coriaceous, for the most part composed of closely compacted interwoven fibrous hyphae 0.8–2.5  $\mu$  in diameter, with thick refractive walls and with lumina capillary or absent, hyaline in water or potassium hydroxide, reddish brown in Melzer's solution, no septa observed, branching irregularly; thin-walled nodulose frequently branched hyphae with clamps scattered in subiculum from which the basidia arise; gloeocystidia absent; basidia at first subglobose, becoming irregularly obpyriform, at maturity ventricose or subventricose below, long cylindrical flexuous above, 25–30  $\times$  5–6  $\mu$ , more or less truncate apically with four straight sterigmata 5–5.5  $\mu$  long; basidiospores subclavate, slightly curved, laterally flattened and attenuate toward base, 12–14  $\times$  2.5–3  $\mu$  sometimes adherent in groups of two to four, walls thin, hyaline, smooth, non-amyloid.

### **Specimens examined:**

**Ontario:** On bark of decaying branches of *Quercus alba* on ground, Peta-wawa Forest Reserve, Chalk River, Ont., Sept. 12, 1939, 14121 type; on stems of *Osmunda Claytoniana*, same locality, Aug. 31, 1941, 17326; on stems of *Rubus* spp. woods W. of Maple, Oct. 4, 1947, 22080, 22081.

**Iowa:** On oak (?) leaves on ground, Iowa City, Aug. 17, 1943, G. W. Martin 4911, TRT.

This species belongs in Section 10 *Trichostroma* of the Bourdot and Galzin classification. They include in this section *Corticium odoratum* (Fr.) B. & G., *C. portentosum* B. & C. and *C. subodoratum* Karst. We would add to this list the species described above, *C. galactinum* Fr. sensu Burt and *C. abeuns* Burt (= *Gloeocystidium ochroleucum* Bres. & Torr.). The latter species had been included by Bourdot and Galzin in a section similarly named in the genus *Gloeocystidium*. All of these species, with the exception of *C. subodoratum*, are characterized by having a coriaceous subiculum made up largely of narrow fibrous hyphae that turn a characteristic reddish brown in Melzer's solution and possess other characteristics that suggest close relationship. *C. subodoratum* Karst. has thick-walled, somewhat fibrous hyphae in the subiculum but these are not comparable to the subicular hyphae in the other species listed and do not give the characteristic reaction in Melzer's solution. For this and other reasons the species is, we believe, quite unrelated. Examination of the type of *C. subodoratum* shows that it is a synonym of *C. rubicundum* Burt.

With this elimination, the species mentioned above all show characters that suggest a relationship with the genus *Vararia* (= *Asterostromella*) and it seems quite probable that ultimately they will be found to form a natural section of that genus or perhaps be united in a separate related genus. Until such time as the species of *Vararia* and their relatives can all be reviewed, it seems best to recognize the section *Trichostroma* of Bourdot & Galzin and to describe the above species in *Corticium*.

***Corticium definitum* sp. nov. (Fig. 4)**

Fructificatio extensa, tenuis, siccitate minute reticulata, glauco-caerulea dein cinerea; subiculum paulum, hyphae basales 3-4  $\mu$  diam., nodoso-septatae, tunicis firmis, partim leviter incrassatis, ad rectam straturam subhymenialem producendum ramosis; cystidia subcylindracea, subflexuosa, 35-50  $\times$  5.5-7  $\mu$  immersa vel parum emergentia; basidia subcylindracea, flexuosa, saepe infra ventricosa, 28-40  $\times$  5.5-8.5  $\mu$ , 4 sterigmata subulata 6.5-8.5  $\mu$  longa gerentia; basidiosporae subcylindraceae vel allantoideae 11-14  $\times$  3-4  $\mu$ , tunicis tenuibus, hyalinis, levibus.

Fructification extensive, forming a thin interrupted often minutely reticulate growth, blue gray when fresh, smoky when dry, margin indeterminate; basal hyphae few, 3-4  $\mu$  in diameter, with clamps, wall thin, firm, or in part slightly thickened, branching at right angles and forming an upright subhymenial layer; gloeocystidia subcylindrical, somewhat flexuous, 35-50  $\times$  5.5-7  $\mu$ , arising in the subhymenial layer or directly from basal hyphae, immersed or protruding slightly; basidia subcylindrical, flexuous, frequently ventricose below, 28-40  $\times$  5.5-8.5  $\mu$ , at maturity extruded beyond the level of the hymenium of immature basidia, bearing four subulate slightly divergent sterigmata 6.5-8.5  $\mu$  long; basidiospores subcylindric to allantoid, 11-14  $\times$  3-4  $\mu$  with minute lateral apiculus, walls thin, hyaline, smooth, non-amyloid.

**Specimens examined:**

**Ontario:** On decorticated, slightly decayed coniferous wood, woods W. of Maple, York Co., Oct. 18, 1942, **17800 type**.

The relationship of this species is probably with the section *Gloeocystidiales* of *Peniophora* in the Bourdot & Galzin classification and to some species of their section *Ceracea* of *Gloeocystidium*. In this species there is no suggestion of colored resinous secretion characteristic of several of the members of those sections. It matters little, at present, whether forms of this sort are included in *Peniophora* or *Corticium*.

**Corticium sulfureo-isabellinum** Litschauer, apud Pilát, Acta Musei Nat. Pragae, 2B: 43. 1940. (Fig. 5)

Fructification broadly and irregularly effused, thin 50–90  $\mu$ , at first soft ceraceous, subgelatinous, sulphurous or somewhat dingy yellowish, becoming indurated, thinly crustaceous, or submembranous, cream, creamy-alutaceous or isabelline to cinnamon buff, adnate, margin gradually thinning out; hymenium smooth, glabrous, continuous, in age frequently becoming cracked; subiculum somewhat indistinct, basal hyphae horizontal, conglutinate, vertical hyphae densely entangled, with clamps at septa, wall thin becoming collapsed and indistinct; gloeocystidia cylindrical to subclavate 35–75  $\times$  5.5–8  $\mu$ , flexuous; paraphyses in hymenium hyphal-like, flexuous, branching, tortuous to subclavate; basidia broadly clavate, tapering from slender hyphal base, 20–40  $\times$  7–8.5  $\mu$ , bearing four subulate, somewhat arcuate or nearly straight sterigmata 8–10  $\mu$  long; spores globose or subglobose, 5.5–7  $\times$  5–6  $\mu$ , slightly compressed laterally with distinct apiculus, hyaline, walls thin, smooth, non-amyloid, multi- or uniguttulate.

**Specimens examined:**

**Ontario:** On bark of *Abies balsamea*, Bear Island, Lake Timagami, Aug. 11, 1937, 11950; R. Biggs, Aug. 26, 1935, 8653; July 28, 1936, 10467; portage to Gull Lake, W. mainland, Lake Timagami, R. Biggs, Aug. 6, 1935, 8475; Aug. 10, 1936, 10466; Sept. 9, 1936, 10468; R. F. Cain, Aug. 15, 1946, 20962; Algonquin Park, Opeongo Lake, R. F. Cain, Aug. 23, 1939, 14246, 14789; Smith Lake, Aug. 24, 1939, 14791; highway near milepost 2, Oct. 13, 1940, 16263; Sturgeon River, near Beardmore, Thunder Bay District, R. F. Cain, Aug. 29, 1944, 19167; Petawawa Forest Reserve, Chalk River, Sept. 12, 1939, 14320; Sept. 5, 1941, 17387.

**Quebec:** On bark of *Abies balsamea*, near Camp Mercier, Laurentide Nat. Park, Aug. 27, 1938, TRT.

**New Hampshire:** On bark of *Abies balsamea*, Bethlehem, July 27, 1936, H. G. Eno, BPI, FP 81330, TRT.

**Austria:** On bark of *Abies pectinata*, Tirol, Kranebittenklamm near Innsbruck, V. Litschauer, Aug. 8, 1929, TRT.

**Czechoslovakia:** On bark of *Abies alba*, Carpatorossia Bily Potok, A. Pilát, Aug. 1935, TRT type, comm. V. Litschauer.

When the manuscript for the present contribution was first written, it contained a Latin description of the above species that had been sent to the writer by the late Prof. V. Litschauer of Innsbruck, Austria. At that time we were under the impression that the species had not been published. We are indebted to Dr. D. P. Rogers for sending quite recently a reference to the publication of the species for Litschauer in a paper by Dr. A. Pilát (4), which had not previously come to our notice.

The history of our interest in this species follows: On Jan. 29, 1936 a specimen of a gloeocystidiolate *Corticium* collected in the Timagami region in the summer of 1935 (TRT 8475), which we had been unable to identify, was sent to Prof. Litschauer for his opinion. Referring to this specimen, he replied, Feb. 17, 1936, as follows:

"Der Pilz, den Sie mir in dem Brief gesendet haben, ist mir schon lange bekannt. Ich habe ihn schon 2 mal in Tirol gesammelt und er ist mir auch von Herrn Dr. Pilát aus der Cechoslovakei zugesendet worden. Es ist der Pilz eine nov. spec., *noch nicht beschriebene Art*. Sie wird demnächst mit anderen *Corticium*arten von mir veröffentlicht werden. Es handelt sich nicht um eine *Gloeocystidium*art, sondern um ein echtes *Corticium*. Ich habe die Art *Corticium sulfureo-isabellinum* genannt. Auch Herr Bourdot, den ich die Art schon vor mehreren Jahren zur Begutachtung sandte, hat sie als eine nov. spec. erklärt. Ich sende Ihnen 2 Proben der Art zum Vergleich und lege auch die von mir entworfene Beschreibung der neuen Art bei."

In the description to which he refers above no mention was made of gloeocystidia. This description, which was in Latin, is the same as the one recently published in Pilát's paper. Three years later, in 1939, a portion of the New Hampshire collection listed above was sent Litschauer and his attention was called to the presence of gloeocystidia, which had also been found consistently present in several Ontario collections made in the meantime, as well as in the two European collections which he had sent (listed above). He replied on June 27, 1939 as follows: "Der Pilz der Kollekte ist jedenfalls meine Art. Gloeocystiden kann ich aber keine finden".

In connection with the description published by Pilát, no mention is made of the North American collections that had been sent to Litschauer. It seems probable that Litschauer had sent Pilát a copy of the same descriptive sheet that he had sent to us in 1936 that had evidently been prepared previous to the receipt of the first American specimen.

Careful study has shown that gloeocystidia are consistently present in all collections of this species that we have examined, and mention of them is included in the English description given above. With this major modification together with a few minor changes and some rearrangement our description is essentially a translation of that furnished by him in 1936 and as published by Pilát. Litschauer's failure to note the presence of gloeocystidia may have

been due to the fact that they are not abundant and could easily be overlooked if only sections were examined. They can be readily demonstrated if well prepared crushed mounts are examined in potassium hydroxide-phloxine.

The presence of gloeocystidia together with the character of the basidia and size and shape of spores bring this species very close to *Corticium (Gloeocystidium) radiosum* Fr. and for a time it seemed doubtful that the two were distinct. Careful comparative study has shown, however, that there are sufficient differences to justify separation. In *C. radiosum* the fructifications are extensive, usually a bright cream yellow and the margin noticeably radiate while in *C. sulfureo-isabellinum* the color is ochraceous or cinnamon buff, and the fruiting body less effused and without radiate margin. In the former the hyphae are typically without clamps and the spores weakly amyloid while in the latter clamps are regularly present and the spores show no evidence of amyloidity. The gloeocystidia of *C. radiosum* are commonly strongly ventricose below, generally slender cylindrical or flexuous above while in *C. sulfureo-isabellinum* they are cylindrical to subclavate, never or rarely ventricose at the base. The former species has a wide host range, occurring commonly on decorticated wood of both conifers and deciduous trees while so far the latter has been collected only on the bark of *Abies* spp.

The drawings for Fig. 5 were all made from the Austrian collection made by Litschauer with the exception of the gloeocystidium second from the right and the two smaller gloeocystidia and the single spore farthest to the left, which were drawn from TRT 16263.

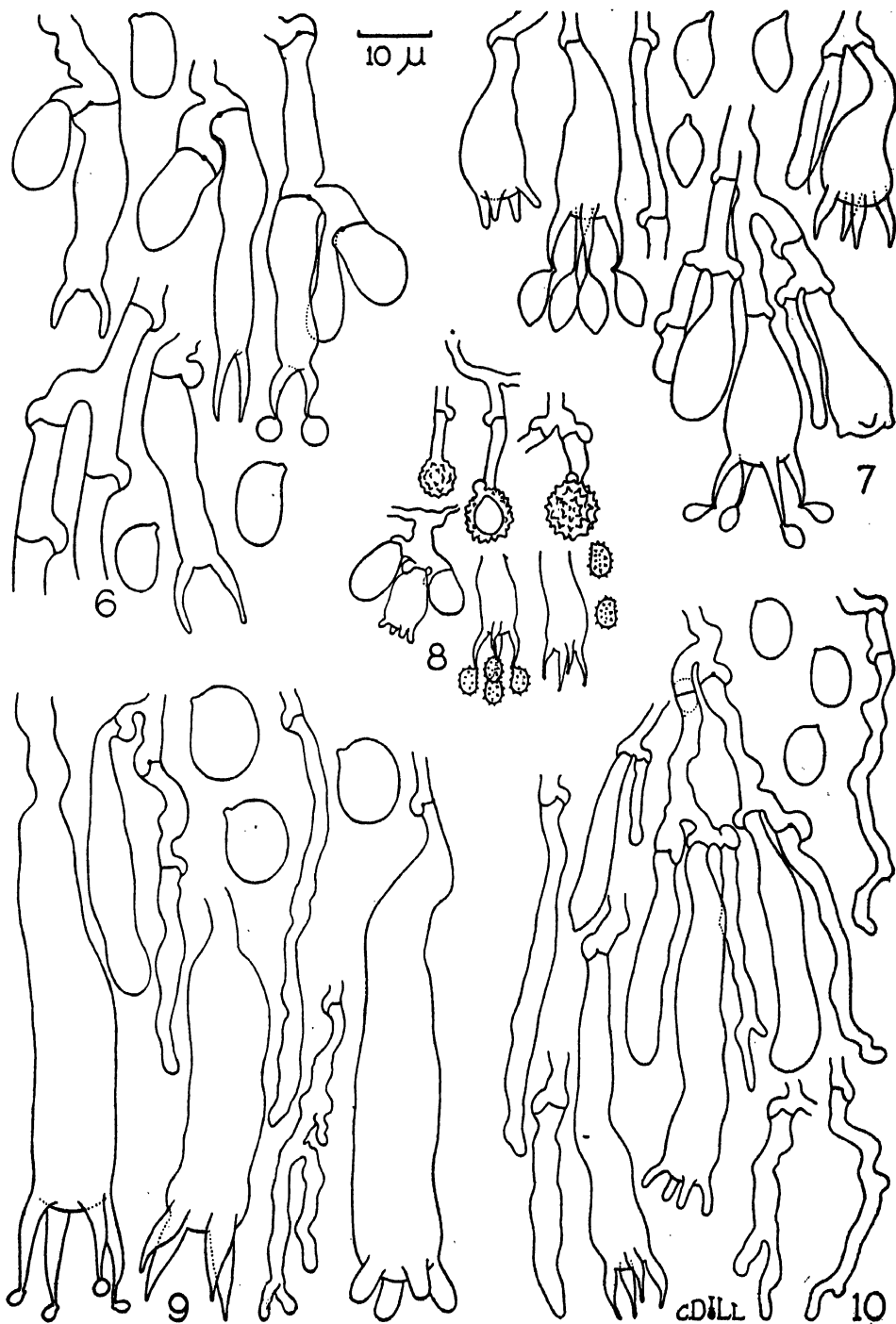
### ***Corticium eximum* sp. nov. (Fig. 6)**

Fructificatio in suborbicularibus coalescentibusque areolis, pruinosa vel poroso-reticulata, alba, 50–70  $\mu$  crassa; subiculum hyphis 3.5–4.5  $\mu$  diam. laxe intricatis, nodoso-septatis; gloeocystidia absentia; basidia 20–25  $\mu$  longa, 5.5  $\mu$  diam., supra cylindracea, infra ventricosa 7–8.5  $\mu$  diam., plerumque 2, raro 3 vel 4 divergentia 6.5–8.5  $\mu$  longa sterigmata gerentia; basidiosporae late ellipsoideae 8–10.5  $\times$  5.5–6  $\mu$ , tunicis tenuibus hyalinis levibus.

Fructifications in irregular, orbicular coalescing patches, pruinose to porous-reticulate, and delicately fibrillose at margins, becoming continuous in centers, conforming to the irregularities of the substratum, white, 50–70  $\mu$  thick in center; a few hyphae 3.5–4.5  $\mu$  in diameter next the substratum are loosely arranged more or less horizontally from which arise upright or ascending branches that branch repeatedly to form a continuous hymenium in the center of the fructifications but more or less interrupted at margin, clamps present at septa, subhymenium made up of shorter and slightly broader cells; gloeocystidia absent; basidia 20–25  $\mu$  long, uniformly cylindric in the upper

FIG. 6. *Corticium eximum*, basidia, spores, and hyphae. FIG. 7. *Corticium reconditum*, basidia and spores. FIG. 8. *Corticium invisitatum*, basidia, spores, and chlamydospores. FIG. 9. *Corticium probatum*, basidia, spores, and paraphyses. FIG. 10. *Corticium notabile*, basidia, spores, and paraphyses.

All figures were drawn with the aid of a camera lucida at a uniform scale, and reproduced at a magnification of approximately 1000 $\times$ .





half and  $5.5\ \mu$  in diameter, ventricose below,  $7-8.5\ \mu$  in diameter, proliferating from base of clamp, bearing usually only two sterigmata  $6.5-8.5\ \mu$  long, becoming divergent, rarely three or four; spores broadly ellipsoid,  $8-9-10.5 \times 5.5-6\ \mu$ , flattened and appearing straight on one side with prominent lateral apiculus, walls thin, hyaline, smooth, non-amyloid.

Specimens examined:

**Ontario:** On bark of lower dead branches of *Pinus Strobus*, woods near Holland River Marsh, 5 miles W. of Aurora, York Co., May 29, 1937, 12916 type.

The relationship of this very distinct species with others of the genus is not at all clear. For the most part the fructifications are associated with old sphaeriaceous fungi. Frequent anastomoses of the basal hyphae were observed and the branches of the basal and upright hyphae often arise from the clamps.

**Corticium reconditum** sp. nov. (Fig. 7)

Fructificatio tenuis, delicata, isabellina, hypochnoidea, siccitate poroso-reticulata; hyphae subiculi,  $1.5-3\ \mu$  diam., tenuem parallelem straturam ramis rectis in quibus hymenium consistit producentes, nodoso-septatae; gloecystidia nulla; basidia late clavata basi antustiora,  $16-20 \times 7.5-8.5\ \mu$ , tunicis fragili rugosa brunneola resinosa incrustatione tectis, 4 gracilia subulata, late divergentia,  $7.5-8\ \mu$  longa sterigmata gerentia; basidiosporae lenticulares, lateraliter compressae,  $8.5-10 \times 4.5-5.5\ \mu$ , tunicis tenuibus hyalinis levibus.

Fructification thin and delicate, isabella color, hypochnoid, becoming minutely porous-reticulate, loosely adherent, margin indeterminate; hyphae of subiculum  $1.5-3\ \mu$  in diameter, forming a thin horizontal layer, giving rise to erect loosely branched hyphae from which the hymenium is formed, septa with clamps; gloecystidia none; basidia cylindrical and hyphalike when first formed, becoming swollen above and at maturity broadly clavate with narrowed base  $16-20 \times 7.5-8.5\ \mu$ , wall frequently covered by delicate rugose brownish resinous incrustation that gives the color to the fructification, bearing four spores on slender subulate widely divergent sterigmata,  $7.5-8\ \mu$  long; basidiospores lenticular, laterally compressed on one side with prominent apiculus  $8.5-10 \times 4.5-5.5\ \mu$ , walls thin, hyaline, smooth, non-amyloid.

Specimens examined:

**Ontario:** On bark of *Pinus Strobus*, Petawawa Forest Reserve, Chalk River, Renfrew Co., Sept. 12, 1939, 17585, type.

The relationship of *C. reconditum* to others in the genus is not clear. Superficially the fructifications resemble those of *Coniophora*. The spores however are entirely colorless and not of the type characteristic of that genus. The color is in part due to a delicate incrustation of brown resinous material, soluble in potassium hydroxide, mostly occurring on the walls of the basidia, that is left after the collapse of old basidia as an obscure brown granular disorganized mass within the hymenium.

The spores are characteristic, sublenticular to subfusiform almost biapiculate, and suggestive of the spores of some strains of *Pellicularia vaga* (Berk. & Curt.) Rogers. The species, however, has no relation to those included in the genus *Pellicularia*.

**Corticium invisitatum** sp. nov. (Fig. 8)

Fructificatio effusa, tenuis, alba, pruinosa vel submembranacea; subiculum paulum, hyphis basalibus paucis, paralleliter currentibus, rectos ramos qui in apice racemus basidiorum vel chlamydosporarum fit ferentibus, hyphis  $2-3\ \mu$  latis, nodoso-septatis, tunicis tenuibus ampulliformibus interdum tumosis; chlamydosporae numerosae,  $5.5-7\ \mu$ , in apice ramorum hypharum productae, globosae vel subglobosae, tunicis incrassatis  $0.8-1.5\ \mu$ , rugosis, hyalinis; basidia oblonga vel cylindracea vel subclavata  $8.5-12 \times 4.5\ \mu$ , 4 gracilibus  $3.5-4.5\ \mu$  longis sterigmatibus; basidiosporae late ellipsoideae vel subreniformes, lateraliter compressae,  $3.5-4.5 \times 2-3\ \mu$ , tunicis hyalinis asperulatisque.

Fructification thin, white, pruinose to submembranous, occurring in broadly effused areas over substratum, loosely adherent, friable, margin indeterminate; subiculum scanty, basal hyphae few, horizontal, giving rise to lateral perpendicular branches that terminate in clusters of basidia or chlamydosporophores; subhymenium with considerable imbedded crystalline material, hyphae  $2-3\ \mu$  broad, walls thin, septa with clamps, ampulliform swellings occasionally present; chlamydosporae numerous,  $5.5-7\ \mu$  in diameter, developing terminally on hyphal branches generally subtended by clamp, globose or subglobose, wall thickened  $0.8-1.5\ \mu$ , rugose, hyaline; basidia oblong to cylindrical or subclavate  $8.5-12 \times 4.5\ \mu$  bearing four slender straight or slightly arcuate sterigmata  $3.5-4.5\ \mu$  long; basidiosporae broadly ellipsoid to subreniform, laterally compressed, broadest at distal end, with minute apiculus,  $3.5-4.5 \times 2-3\ \mu$ , wall hyaline, asperulate, non-amyloid.

Specimens examined:

**Ontario:** On duff and debris on ground in old shack, S.E. of Hatchley, Brant Co., Sept. 3, 1943, R. F. Cain, 18792 type.

A member of Bourdot & Galzin's Section 11 Humicola of *Corticium*, this species is unique because of the presence of thick-walled spores, termed chlamydosporae in the above description, which apparently readily become detached and function as conidia. The nature of the sculpturing on the outer wall of these spores is difficult to determine but there is some evidence that the rugose markings may be in the nature of spirally arranged ridges not well indicated in the drawings of Fig. 8.

The genus *Phlebiella* Karst., as noted in the introduction, is available for this group of species. Until the group as a whole can be adequately reviewed it seems best to continue to recognize the section Humicola and to describe new forms in *Corticium*.

**Corticium probatum** sp. nov. (Fig. 9)

Fructificatio effusa, crenea vel eburneo-citrula, tenuis,  $80\ \mu$ , siccitate membranacea; subiculum, hyphis  $2-3\ \mu$  diam. laxe intricatis, nodoso-septatis; gloeocystidia nulla; paraphyses velut ramosae flexuosaeque hyphae praesentes; basidia magna,  $40-65 \times 10-12\ \mu$ , subcylindracea vel late clavata, subflexuosa, 4 subulatis subarcuatis  $9-12\ \mu$  longis sterigmatibus; basidiosporae ovoideae vel subglobosae,  $9-12 \times 8.5-10\ \mu$ , apiculo laterali prominente lateraliter depressae, tunicis hyalinis tenuibus levibus.

Fructification broadly effused, cream to ivory yellow, thin,  $80\ \mu$ , smooth, soft membranous when dry, somewhat separable, margin determinate, not differentiated or scarcely so as a narrow delicately fibrillose white border; subiculum of loosely interwoven hyphae  $2-3\ \mu$  in diameter, septa with clamps, rosettes of crystals imbedded in subiculum; gloeocystidia none; simple or

branching flexuous hyphae extending into hymenium as paraphyses; basidia large,  $40-65 \times 10-12 \mu$ , subcylindric to broadly clavate, slightly flexuous, narrowed at base, extruded beyond level of hymenium when mature, contents conspicuously guttulate, bearing four subulate slightly arcuate sterigmata  $9-12 \mu$  long; basidiospores ovoid to subglobose,  $9-12 \times 8.5-10 \mu$ , depressed on one side with prominent lateral peglike apiculus, walls colorless, thin, smooth, non-amyloid.

Specimens examined:

**Ontario:** On decayed coniferous wood, woods W. of Maple, York Co., Oct. 21, 1939, 17589 type.

*C. probatum* is another species with large *Aleurodiscus*-like basidia, the relationship of which with other species is not clear. The large basidia, hypha-like paraphyses and the characteristic, rather large spores, form a combination of characters that serve to separate it from other described species.

**Corticium notabile** sp. nov. (Fig. 10)

Fructificatio albidia vel eburneo-citrula, subceracea, siccitate subindurata, in panniculis elongatis producta, dein rimosa, adnata, usque ad  $650 \mu$  crassa, margine determinato; subiculum obscure stratosum, hyphis fere rectis,  $1.5-3.5 \mu$  diam., nodoso-septatis, tenuiter tunicatis compositum; basidia clavata  $40-50 \times 7-8 \mu$ , quattuor subulata sterigmata  $7.5-8.5 \mu$  gerentia; basidia cum paraphysibus velut hyphis, interdum subacuminata, saepe nodulosa, interdum furcata, vel obtusis furcis; basidiosporae ovoideae  $7-9 \times 4.5-6 \mu$ , tunicis tenuibus hyalinis levibus.

Fructification whitish to ivory yellow, subceraceous, somewhat indurated when dry, occurring in elongate irregular patches, to  $650 \mu$  thick, becoming rimose, adherent; margin determinate and undifferentiated; subiculum not sharply differentiated into layers, basal horizontal portion of hyphae with thickened, highly refractive, somewhat gelatinized walls from which arises an indistinctly stratose portion of vertical hyphae that ultimately form the hymenium; upright hyphae  $1.5-3.0 \mu$ , thin-walled, with clamps, interwoven with granular-appearing collapsed hyphae; basidia clavate, somewhat flexuous  $40-50 \times 7-8 \mu$ , bearing four subulate nearly straight sterigmata,  $7.5-8.5 \mu$  long, accompanied by hyphalike paraphyses sometimes subacuminate, often nodulose, occasionally furcate, or with stout prongs; basidiospores ovoid,  $7-9 \times 4.5-7 \mu$  slightly compressed laterally with minute apiculus, walls thin, hyaline, smooth, non-amyloid.

Specimens examined:

**Ontario:** On decorticated wood of *Picea mariana*, Ko-ko-ko Bay, Lake Timagami, July 19, 1938, 13682, 13689; R. F. Cain 13687; Aug. 3, 1939, 15005, 14911, type; July 21, 1943, 18668; Petawawa Forest Reserve, Chalk River, Sept. 2, 1941, 18060; on *Pinus Banksiana*, Constance Bay, Ottawa River, June 30, 1939, 15167; Sturgeon River, near Beardmore, Thunder Bay District, R. F. Cain, Aug. 30, 1944, 19169; on *Thuja occidentalis*, woods S. of Aurora, York Co., Oct. 1, 1939, 16548; near Friday's lodge W. shore of Lake Timagami, R. F. Cain, Aug. 12, 1939, 14969; on coniferous wood, Bear Island, Lake Timagami, R. Biggs, Aug. 10, 1935, 16754; Aug. 16, 1935, 8215; N. of Mt. Albert, York Co., Oct. 28, 1938, 13688.

**Nova Scotia:** On deciduous wood, U. Brookside, Colch Co., July 4, 1935, L. E. Wehmeyer 385, TRT.

**Manitoba:** On *Pinus Banksiana*, Victoria Beach, I. Mounce & G. R. Bisby, June 22, 1935, OTB F6324, TRT; Sandilands Forest Reserve, G. R. Bisby, Oct. 4, 1936, OTB F7056, TRT; on *Picea*, 13 miles E. of Beausejour, G. R. Bisby and A. H. R. Buller, Sept. 29, 1935, OTB F6431, TRT.

**Connecticut:** On *Castanea dentata*, N. Bloomfield, H. G. Eno, June 18, 1937, BPI, FP 82347, TRT; Black Rock State Forest, Watertown, H. G. Eno, Aug. 23, 1939, BPI FP84825, TRT.

**California:** On coniferous wood, near summit of old LaHonda Rd., San Mateo Co., Nov. 13, 1942, M. Doty 3388, TRT.

This rather conspicuous species has proven so common that it seems surprising that it has not been described previously. All of the collections recorded from Ontario were made on coniferous wood. We have included three collections from Nova Scotia and Connecticut occurring on wood of deciduous trees that seem to be the same. The gross characters of the fructifications together with the presence of abundant hyphal paraphyses and other microscopic details serve to distinguish this species from others with which we are familiar.

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## FASCIATION OF SWEET PEAS<sup>1</sup>

BY E. H. GARRARD<sup>2</sup>

### Abstract

A description is given of fasciation in a sweet pea plant, from which a strain of *Agrobacterium tumefaciens* (Smith and Townsend) Conn was isolated. Germinated and nongerminated sweet pea seed inoculated with the organism were grown in agar, sand, and soil. Fasciation, dwarfing, swollen roots and stems as well as yellowed and crinkled leaves were produced in most of the plants grown in agar and sand. Fewer plants grown in soil were affected. Microscopical examinations revealed organisms in fasciated areas, roots, and stems. The organisms were recovered in each case by plating with Congo red agar.

Fasciation was not produced in garden pea plants although some plants were dwarfed. Typical galls were not produced on stems of sweet and garden pea plants by needle inoculations. A description of the organism is given and certain features of its action on sweet pea plants are discussed.

### Introduction

Fasciation of sweet peas has been reported on various occasions and different organisms have been claimed to be the cause of the disease. Brown (1) described fasciation of sweet pea plants from New Jersey and New York States and of garden pea plants from Maryland and Virginia caused by a weak or highly specialized strain of *Agrobacterium tumefaciens*. Sweet pea fasciation is reported by Muncie and Patel (4) to be caused by *Agrobacterium tumefaciens*, certain fungi, and other environmental factors. Tilford (7) isolated *Xanthomonas fasciens* as the cause of fasciation and failed to produce typical fasciation in sweet peas by inoculation with the crown gall organism. Lacey (2) isolated an organism similar to *Xanthomonas fasciens* associated with fasciation of sweet pea and other plants.

This paper deals with fasciation of sweet peas caused by an organism resembling *Agrobacterium tumefaciens* and presents certain features to which little reference has been made in the literature.

### Description of Original Specimen

The original sweet pea plant submitted for examination was 10 in. in height; the stem was spindly and the leaves were dwarfed, yellow, and somewhat shrivelled. A leafy gall-like structure, composed of distorted leaves and shoots, was situated at the crown of the plant. It was compressed into an irregularly shaped mass approximately 2.5 cm. in diameter. The interior was light green in color and quite firm.

Enquiries revealed that 40 plants, representing one-third of the crop, showed similar symptoms of dwarfing, with spindly stems and general lack

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of vigor and color. The sweet pea plants were grown from imported seed and consisted of a mixture of 12 early or winter flowering varieties. They were sown in loam composed of rotted sod and later transferred to a permanent bed in the greenhouse, the soil in which was composed of a mixture of muck, rotted sod, and well rotted manure. No other legumes previously had been grown in the same soil.

### Isolation of Causal Organism

Microscopical examination of various portions of the abnormal tissue revealed the presence of numerous, medium-sized, Gram-negative rods of various shapes. These included curved forms, rods with swollen ends, y forms, and other bacteroidal shapes similar to those described by Smith (6). Sections of the tissue were macerated in sterile water and plated with Congo red agar. After seven days' incubation at 25° C. (77° F.) numerous dark red colonies appeared on all the plates. Representative colonies were transferred to slants for further observation. Microscopical examinations of the cultures showed the same rod-shaped organism without involution forms; star-shaped grouping, however, was quite common.

### Artificial Inoculations

#### *A. Agar Cultures*

Mixed, winter flowering sweet pea seeds were sterilized by submersing them in alcohol for one minute, followed by mercuric chloride 1 : 1000 for seven minutes under vacuum, with several changes of sterile water. The seeds were germinated on moist blotting paper in sterile Petri dishes. They then were transferred with flamed forceps to 2 in. diameter test tubes containing modified Crone's agar. A drop of suspension of the isolated organism was deposited on each seed. The test tubes were wrapped in brown paper and maintained for a period of approximately six weeks. Modified Crone's solution was added from time to time.

Twenty-six of 32 plants developed fasciation or other signs of abnormality. First signs of fasciation appeared about the end of the third week and from that time on, the plants exhibited various effects of the action of the organism. These included dwarfing, thin or swollen roots, lack of roots, and crinkled, yellowed leaves. These are illustrated in Plate I. Fig. 1 shows the development of fasciation on a plant in a test tube at approximately four weeks. Fig. 2 shows the tallest of the fasciated plants (10 in.) with an uninoculated control plant. Fig. 3 presents an enlarged view of the fasciated area of the plant shown in Fig. 2. In Fig. 4 is shown a six-weeks-old plant that developed a poor root system and no top. Fig. 6 shows an enlarged view of the top of the plant shown in Fig. 2 where the leaves are crinkled and the stem is swollen at one point.

Microscopical examinations of swollen portion of the stem, fasciated area at the crown, and of various sections of the roots revealed characteristic

bacteria in great numbers. No difficulty was experienced in recovering them from all three locations on Congo red agar.

### B. Sand Cultures

The experiment was repeated with sweet pea seed grown in sand. Washed quartz sand was sterilized in shallow trays at 350° F. for 10 hr. and placed in wide mouthed 3 in. glass jars. The seeds were sterilized as before and germinated, as well as nongerminated, seeds were deposited 1 in. below the surface. They were inoculated by adding a drop of suspension of the organism on the seed before covering over. Controls were included as well as seeds inoculated with a true strain of *Agrobacterium tumefaciens* procured from Dr. M. P. Starr, Hopkins Marine Station of Stanford University. The jars were wrapped in brown paper and held for six to eight weeks. Modified Crone's solution was added daily.

All but one of 18 inoculated seeds developed plants with fasciation or other signs of abnormality. Several seedlings attained a height of 1 in. and died. Examination of such seedlings revealed countless numbers of organisms in all parts of the shoots. A few plants developed fairly normal tops, but possessed two or more dwarfed or swollen shoots arising from a gall-like area at the crown. Others failed to attain a height of more than 2 to 4 in. and exhibited flattened or swollen shoots with few or no roots. Examples of this latter type are shown in Fig. 7.

Fig. 5 shows the fasciated areas of two plants that contained nodular swellings on the roots. Although dark in color, they somewhat resembled *Rhizobium* nodules but contained the same organisms found in fasciated shoots, roots, and stems of other plants. Moreover, organisms isolated from these swellings produced typical fasciation in other seedlings inoculated at a later date.

No sign of fasciation or abnormality was found in the seedlings inoculated with the true strain of *Agrobacterium tumefaciens* and the plants compared favorably with the controls, which attained an average height of 18 to 20 in. No difference could be found between germinated and nongerminated seed as plants from both groups developed typical symptoms.

### C. Soil Cultures

Sterilized germinated and nongerminated seeds were sown in 4 in. pots containing sterilized garden soil. More than 150 seeds were used, four to each pot. Seeds were inoculated with the isolated organism and others with the true strain of the crown gall organism. They were held for eight weeks.

The results were not so conclusive as in the preceding experiments. Approximately 50% of the plants were affected and dwarfing and yellowing of the plants were more common than gall-like areas at the crown. Certain plants died when they were 2 to 3 in. high and others never attained a height of more than 5 in. Fig. 8 shows three of these plants at six weeks of age. The control plants attained an average height of 14 to 16 in. The plants inoculated



FIGS. 1 to 6. *Fasciation of sweet peas from inoculated seed.* 1. Fasciation of four-weeks-old plant in test tube. 2. Six-weeks-old fasciated plant and control, grown on agar. 3. Enlarged view of fasciated area of plant shown in Fig. 2. 4. Six-weeks-old plant without top, grown on agar. 5. Fasciated area of plants grown in sand. 6. Enlarged view of top of plant shown in Fig. 2.





FIGS. 7 to 9. Fasciation of sweet pea plants from inoculated seed. 7. Dwarfed fasciated plants grown in sand. 8. Dwarfed forms grown in soil. 9. Garden pea stem showing swelling as a result of needle inoculation.

with *Agrobacterium tumefaciens* showed no signs of the disease and were as healthy and vigorous as the controls.

Microscopical examinations of the stems of the plants shown in Fig. 8 revealed typical organisms that were recovered on plating.

### Examination of Soil

Although it was not possible to obtain any of the original sweet pea seed, a representative sample of soil in which the original plants were grown was obtained. Attempts to isolate the organism directly from the soil resulted in failure. Sterilized germinated seed grown in the soil resulted in only one fasciated and dwarfed plant being obtained, from which typical organisms were recovered.

### Garden Pea Inoculations

Garden pea seeds were sterilized, germinated, inoculated, and sown in sterile sand. They were held until the controls formed pods. No cases of fasciation developed, but a few of the plants never attained a height of more than 4 in. The roots on these plants were swollen, the stems were spindly, and the leaves slightly yellowed. Organisms were recovered from the roots and lower parts of the stems.

### Gall Production

In an attempt to determine whether the organism was capable of producing typical galls on the upper parts of the plant, both sweet and garden pea control plants were inoculated by needle in various portions of the main stem and petioles. Inoculations also were conducted with the true crown gall organism. While the latter organism produced definite galls about the size of a pea, only one somewhat inconclusive swelling was produced by the isolated organism on the upper part of a garden pea plant. This is shown in Fig. 9 and it somewhat resembles the swelling found on the upper part of the stem shown in Fig. 6, which resulted from seed inoculation. Organisms were recovered from this swelling on Congo red agar.

### Description of the Organism

Repeated biochemical and cultural tests with the organism have revealed that it differs only slightly from most descriptions of *Agrobacterium tumefaciens*. Therefore it would appear that the organism is similar to that found by Brown (1), although she does not describe the organism in detail, and that it is a weak or highly specialized form of the crown gall organism. The following is a description of the organism.

A medium sized rod, measuring  $0.5 - 0.8 \times 1.5 - 3.0 \mu$ , motile by one to two polar flagella; no spores; capsules; Gram-negative; grouped singly and in pairs; bacterioid forms found in carbohydrate media. Aerobic; nutrient agar colonies—grayish-white, circular, raised, glistening, entire, becoming viscid; Congo red agar—raised, circular, glistening, and strongly

absorbs the dye; nutrient broth—moderately turbid with suspended filaments, fragile pellicle; gelatin—not liquefied; nitrates—not reduced; indol—not produced;  $H_2S$ —not produced. Growth in Uschinsky's medium, no growth in Cohn's solution. No gas in carbohydrate media but slight acid in arabinose, galactose, mannitol, and mannose. B.C.P. milk—neutral to alkaline, no coagulation but definite serum zone; optimum temperature—25° to 30° C.

### Discussion

The symptoms of fasciation and prolepsis of sweet pea plants here described generally resemble descriptions of the disease by other workers. Certain differences have been observed, however, that are of particular interest. In the first place Smith (5) refers to the almost complete sterility of fasciated shoots in plants, and Brown (1) reports that microscopical examination of fasciated shoots failed to reveal the presence of microorganisms, although they were isolated on plating. Also, it has been generally observed that primary inoculations with gall-forming strains into certain hosts may result in secondary galls in which no typical organisms are found.

In this series of experiments there has not been any difficulty in demonstrating countless numbers of characteristic organisms in fasciated shoots, stems, or roots and subsequent agar plates in high dilutions have been crowded with colonies. While in some plants, more sturdy than others, the organisms were localized in the fasciated areas, most of the stunted and dwarfed plants revealed organisms in the extreme tips of the shoots and in the swollen root ends. The fact that many distorted seedlings with organisms distributed throughout the tissues died quite rapidly demonstrates the unusual invasive activity of this particular strain. Also, such activity no doubt accounts for the pronounced dwarfing and sickly appearance of more mature plants.

The majority of cases of fasciation in plants by artificial inoculation have been effected by needle inoculation (1, 3, 5). In sweet peas, Brown (1) allowed seeds to germinate and then made needle inoculations into the tender shoots. With the exception of needle inoculations into stems to try to produce galls, all inoculations in these experiments were made by adding a suspension of the organism directly to germinated or nongerminated seeds.

Among numerous descriptions of fasciation in plants there is little or no mention of swellings on roots or stems. Swollen roots and nodular protuberances, as shown in Figs. 3 and 5, were quite common in these experiments. Swollen areas on the stems, as shown in Fig. 6, were present but not so commonly encountered. Examination of each swelling always revealed characteristic organisms.

It is not known how common sweet pea fasciation is in Ontario, nor could it be determined whether the trouble was seed- or soil-borne. The above findings, however, do corroborate the fact that organisms other than *Xanthomonas fasciens* are capable of causing fasciation and abnormal development in sweet pea plants.

### Acknowledgments

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# STUDIES ON THE METABOLISM OF *STREPTOMYCES GRISEUS* IN RELATION TO THE PRODUCTION OF STREPTOMYCIN<sup>1</sup>

BY H. M. EISER<sup>2</sup> AND W. D. MCFARLANE<sup>3</sup>

## Abstract

A synthetic medium inducing high streptomycin production was evolved by studying growth factor and nitrogen requirements of the mold *Streptomyces griseus*. It was concluded that mycelium growth and streptomycin production do not necessarily parallel each other. Histidine appeared to affect both streptomycin and mycelium formation and was essential in any amino acid combination to induce either a high mycelium or streptomycin yield. Valine was shown to stimulate streptomycin synthesis and aspartic or glutamic promoted only mycelium production. Experiments were done to show which metabolic changes in the medium could be associated with growth and which with the antibiotic production.

The effect on the mold of high concentration of sodium chloride was investigated. It was found that by reducing the amount of salt in the nutrient media, the greater part of the streptomycin could be recovered from the mycelium instead of the medium. It appears that the antibiotic is a product of intracellular synthesis, since ions of the lyotropic series affecting the permeability of the cellular membrane affect the distribution of the antibiotic between medium and mycelium.

## Introduction

It has now been definitely established that a considerable number of the actinomycetes isolated from soils or other natural substances have the capacity to inhibit the growth of microorganisms. Streptomycin (8) is the most important antibiotic so far obtained from actinomycetes. Its antibacterial spectrum includes both Gram-positive and Gram-negative organisms; moreover in pure form its toxicity is low. With a view of increasing the yield of the antibiotic, research was directed along these main lines:

- (a). To isolate more active strains, giving higher antibiotic titers.
- (b). To study the specific nutrient requirements for the synthesis of streptomycin.
- (c). To develop better methods of isolating the antibiotic from the medium.

The two latter lines of investigation went hand in hand, because the complexity of the media as regards organic constituents made the isolation of streptomycin difficult; the recovery was poor and the isolated product was colored and often toxic at the dosage used for clinical treatment.

The medium first used for the production of the antibiotic was that of Waksman and co-workers (5). Other media (7, 3, 9) have been developed, but none of these has been a defined, synthetic medium. Neither the mechanism

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involved in the production of the antibiotic, nor the nutrients required for its synthesis are as yet known but it appears that some of the active constituents of meat extract (Waksman's medium) are inorganic (7).

This study was undertaken to develop new media, preferably of known composition, to study the metabolism of the organism when grown in these media, to elucidate the mechanism of streptomycin production, and finally to investigate the observation that sodium chloride has an effect on the distribution of streptomycin between medium and mycelium.

### Procedure

The strain *Actinomyces griseus* 10 (Waksman)\* was used throughout the investigation. The organism was grown in test tube soil culture and slants (nutrient agar) for experimental work were inoculated from the soil culture. Submerged culture was used throughout by agitating test tubes (25 × 200 mm.) in a slanting position. This method offered several advantages, e.g., the small volume gave greater economy, especially when working with amino acids, easier handling, a more uniform inoculum (one inoculation slant served 40 test tubes.) The tubes each containing 10 ml. of medium were autoclaved at 15 lb. for 15 min. They were inoculated with 0.2 ml. of spore suspension (10 ml. of sterile water per test tube slant, spore suspension) and placed at a slant of 23 degrees horizontal. This angle proved best, giving a yield of streptomycin equivalent to that obtained with 50 ml. of medium in 250 ml. Erlenmeyer flasks on the same shaker and within the same period of time. The shaker had a backward and forward motion (84 strokes per minute) and was in a constant temperature (26.6° C.), constant humidity room (75% relative humidity). When streptomycin production reached a maximum (assay), the tubes were removed from the shaker, and the original volume of the medium was restored to compensate for loss due to evaporation. The mycelium was separated by centrifuging, the medium placed in the refrigerator, and samples withdrawn for analysis.

To estimate the streptomycin potency of the medium, a technique based upon the assay of penicillin by Foster and Woodruff (1) was employed, taking into consideration the special precautions observed in the assay of streptomycin as outlined by Loo and co-workers (4).

Glucose was determined by Hanes's (2) modification of the Hagedorn Jensen method. Hydrogen ion concentration was measured with a Hellige pH meter. The media were all adjusted to pH 6.8 to 7.0 before each run. Histidine was determined according to Macpherson's (5) modification of the Hanke-Koessler reaction. Ammonia was measured by the method of Van Slyke and Cullen (11) using 20% sodium carbonate and aerating for at least four hours and usually overnight to ensure complete distillation. Amino nitrogen was determined by the method of Van Slyke as outlined in the

\* Kindly supplied by Dr. G. A. Grant, Ayerst, McKenna and Harrison Ltd., Montreal, Que.

*A.O.A.C. Book of Methods* (10). The weight of mycelium was determined by filtering or centrifuging, washing thoroughly and weighing after drying at 80° C.

## Results

### NITROGEN REQUIREMENTS

Before studying in detail the nitrogen requirements of *Streptomyces griseus*, it was thought advisable to examine the growth factors as to their effect on streptomycin production. The basal medium was made up as follows:

Glycine.....	0.4%	(equal in nitrogen content to 0.5% peptone)
Glucose.....	1.0%	
Potassium nitrate.....	0.05%	
Sodium chloride.....	0.5%	
Sodium dibasic phosphate $\times 12 \text{ H}_2\text{O}$ ...	0.18%	
Magnesium chloride $\times 6 \text{ H}_2\text{O}$ .....	0.05%	
Iron chloride $\times 6 \text{ H}_2\text{O}$ .....	0.003%	
Copper sulphate.....	0.001%	

The growth factors were added individually and streptomycin and mycelium production were measured. Inositol increased the yield of both streptomycin and mycelium, riboflavin and nicotinic acid seemed to have some effect on stimulating streptomycin production but none on mycelium formation. Folic acid, pyridoxine, pantothenic acid, thiamine, and *p*-aminobenzoic acid showed no stimulatory effect whatsoever. The results, even with inositol, were significantly lower than those obtained with Waksman's medium, thus indicating the need for a detailed study of the nitrogen source.

As pointed out by Waksman (12) actinomycetes develop most readily if complex nitrogenous substances such as proteins are included in the medium. All naturally occurring amino acids were incorporated individually into the basal medium mentioned, in amounts so that the total nitrogen content was equal to that of a 0.5% solution of peptone. An exception was made in the case of histidine and tryptophane since the utilization of the ring nitrogen was in doubt. The efficiency of the individual acids in inducing mycelium and streptomycin production was not confined to any particular group. Histidine gave the best antibiotic production. The optimum ratio of histidine to glucose was next studied. Mycelium production was estimated visually, a value of 5 representing the optimum growth for that experiment (Table I).

To obtain still higher yields of the antibiotic, combinations of amino acids were tested in which each of the amino acids contributed one-third of the total nitrogen equivalent to that in a medium containing 0.5% peptone (Table II).

All combinations containing valine proved superior. The histidine-valine-arginine medium was studied further by varying the concentration of each. If the amount of histidine was kept constant and the valine concentration varied, there was a direct correlation between valine concentration and streptomycin production but not with mycelium formation. The concentration

of histidine must be above a certain level if this is to be observed. If the amount of valine was constant and the amount of histidine varied, there was a direct correlation between histidine concentration and streptomycin and mycelium production. Arginine seemed to have little effect on the production of streptomycin or mycelium.

TABLE I

THE EFFECT OF THE CARBON-NITROGEN RATIO (GLUCOSE HISTIDINE) ON THE PRODUCTION OF STREPTOMYCIN AND MYCELIUM

Medium*		Zone of inhibition, mm.	Mycelium production
Histidine monohydrochloride, %	Glucose, %		
0.1	1.0	19.0	1
0.2	1.0	20.5	3
0.5	1.0	23.0	4
1.0	1.0	24.5	5
2.5	1.0	22.0	4
0.7	1.5	24.5	5
0.7	1.3	25.5	5
0.7	1.0	23.5	5
0.7	0.7	22.0	4

\* In addition the medium contained inorganic salts and 0.0005% inositol.

TABLE II

THE EFFECT OF COMBINATIONS OF AMINO ACIDS ON STREPTOMYCIN PRODUCTION

Addition to basal medium*			Zone of inhibition, mm.
0.23% histidine,	0.23% valine,	0.22% proline	24.0
0.23% histidine,	0.1% arginine,	0.33% tyrosine	20.0
0.23% histidine,	0.23% threonine,	0.18% lysine	22.0
0.23% histidine,	0.1% arginine,	0.23% valine	26.0
0.23% histidine,	0.23% proline,	0.33% tyrosine	20.5
0.23% histidine,	0.23% valine,	0.23% threonine	25.5
0.23% histidine,	0.23% proline,	0.18% lysine	23.0

\* Basal medium: 1.3% glucose, inorganic salts, inositol.

The medium containing 0.23% histidine, 0.23% valine, and 0.1% arginine was compared with Waksman's medium and the zone of inhibition expressed in 'S' units of streptomycin.\* Waksman's medium produced 180 'S' units, whereas the synthetic medium gave up to 250 'S' units per ml. of medium.

\* An 'S' unit of streptomycin is the amount of the antibiotic that will inhibit the growth of *Escherichia coli* in 1 ml. of nutrient broth.



STUDIES ON THE METABOLISM OF *Streptomyces griseus* GROWN ON CHEMICALLY DEFINED MEDIA

In this investigation it was observed that replacing valine with aspartic acid greatly enhanced the mycelium production but lowered significantly the yield of the antibiotic. When the metabolism of *Streptomyces griseus* was studied, using a complex medium as substrate, it was found (13) that maximum streptomycin production coincided with maximum mycelium formation. It was hoped that a detailed study of two synthetic media, one supporting the streptomycin production and the other mycelium production, would show which metabolic changes were associated with growth and which with the formation of the antibiotic.

The two media had the following composition:

Medium I	Medium II
0.23% Valine	0.23% Aspartic acid
0.23% Histidine	0.23% Histidine
0.10% Arginine	0.10% Arginine
1.30% Glucose	1.30% Glucose
Inorganic salts	Inorganic salts
0.0005% Inositol	0.0005% Inositol

The results are presented in tabular form, the percentage is expressed relative to the highest value obtained for any one variable with both media, which is rated as 100% (Tables III and IV).

TABLE III

METABOLIC CHANGES DURING GROWTH IN A HISTIDINE-VALINE-ARGININE MEDIUM

Analysis of medium	Before sterilization	After sterilization	Duration of fermentation, days					
			1	2	3	4	5	7
pH	6.90	6.90	6.90	6.80	6.75	6.70	6.85	8.50
Residual glucose (mgm. per ml.)	12.2	12.4	12.4	11.3	10.6	8.8	6.25	0.60
Mycelium production (mgm. per 20 ml.)	—	—	—	5	7	20	48	65
Ammonia (mgm. per 10 ml.)	0.68	0.88	0.89	0.88	0.90	0.90	0.91	5.32
Residual histidine (mgm. per ml.)	2.15	2.15	2.15	2.15	1.95	1.90	1.68	0.523
Streptomycin production ('S' units per ml.)	—	—	—	—	—	40	230	80

The metabolism in the aspartic acid medium was characterized by a high production of mycelium, rapid uptake of glucose and histidine, ammonia production, increase in alkalinity, and a low yield of streptomycin. The lag phase was only about 24 hr. and then changes occur that are characteristic of rapid growth. The results obtained resemble those reported by Waksman

TABLE IV

METABOLIC CHANGES IN THE HISTIDINE - ASPARTIC ACID - ARGININE MEDIUM

Analysis of medium	Before sterilization	After sterilization	Duration of fermentation, days					
			1	2	3	4	5	7
pH	6.90	6.80	6.80	6.80	7.10	7.30	7.70	9.10
Residual glucose (mgm. per ml.)	12.2	12.4	12.2	11.3	9.4	6.0	1.25	0.25
Mycelium production (mgm. per 20 ml.)	—	—	—	11.5	29.1	55.5	85.0	80.0
Ammonia (mgm. per 10 ml.)	0.886	1.06	1.06	1.86	2.40	2.94	4.17	6.60
Residual histidine (mgm. per ml.)	2.15	2.15	2.15	1.88	1.10	0.55	0.06	0.02
Streptomycin production						10	18	30

and co-workers (13) using complex media. It can be concluded that the changes are all characteristic of growth and can not be correlated with the streptomycin producing mechanism. Histidine and glucose are consumed at approximately the same rate and practically disappear at the time mycelium production is maximal. The alkalinity increases steadily and is directly related to the corresponding rise in ammonia. Streptomycin production is low and bears no relation to mycelium production.

Up to the fifth day, the metabolism of the valine medium was characterized by less mycelium, slower uptake of glucose and histidine, practically no formation of ammonia, no change in hydrogen ion concentration but a high yield of streptomycin. The lag phase was of longer duration and although mycelium production was rapid from the third day on, the concentration of ammonia and the hydrogen ion concentration remained constant. It was not until the fifth day that the over-all changes resembled those in complex media. It is doubtful whether any of these factors are associated with the streptomycin producing mechanism. It can be tentatively concluded that the development of an alkaline reaction in complex media has no effect on the production of streptomycin.

It was possible to replace aspartic acid with glutamic or even with succinic acid plus nitrate and still obtain the same results as with aspartic acid, namely high mycelium but low streptomycin production. From these data, it is suggested that the metabolism of the aspartic acid, involving the aspartase-transaminase reaction, increases mycelium production at the cost of streptomycin, whereas another, as yet unexplained, mechanism operates in the case of valine. It appears as if the mycelium production does not parallel streptomycin production if the transamination systems are predominantly involved in the synthesis of mycelial protein.

## STUDIES ON THE DISTRIBUTION OF STREPTOMYCIN BETWEEN MYCELIUM AND MEDIUM

It was shown that without sodium chloride Waksman's medium gave a low yield of streptomycin (8). Furthermore, all media so far developed that gave high streptomycin production contained sodium chloride (9, 3). One author reported that the chloride ion could be replaced by the sulphate ion, but the sodium ion could not be replaced by magnesium (7).

Two media were prepared, i.e., Waksman's medium with and without sodium chloride, and tested for streptomycin and mycelium production. The mycelium was separated by centrifuging and a difference in appearance was observed. Mycelium from the sodium chloride medium was dense in appearance, occupying only a small volume in the centrifuge tube, and had a dark color. The mycelium grown on the sodium chloride deficient medium was light colored, fluffy in appearance, and occupied a volume about two and one-half times that of the mycelium from the sodium chloride. When washed, dried, and weighed, however, the amount of mycelium produced in both cases was about the same. However, with the sodium chloride media the streptomycin content of the medium was higher.

A study of the metabolism of *Streptomyces griseus* in both media was carried out to elucidate the function of the high concentration of sodium chloride in Waksman's medium. The results with both media are given in one table, Table V, since there was no significant difference attributable to the influence of sodium chloride. However, as reported previously, the yield of strepto-

TABLE V

METABOLIC CHANGES IN WAKSMAN'S MEDIUM WITH AND WITHOUT SODIUM CHLORIDE

Period of fermentation	pH*	Streptomycin ('S' units per ml.)		Glucose (mgm. per ml.)*	Ammonia (mgm. per 10 ml.)*	Mycelium (mgm. per 10 ml.)*	Amino nitrogen (mgm. per 10 ml.)*
		With NaCl	Without NaCl				
0	6.9	—	—	9.5	0.15	—	2.4
1 day	7.5	—	—	9.0	0.29	28	2.0
2 days	7.5	130	25	5.0	0.27	104	1.0
3 days	7.9	180	30	1.7	0.30	82	1.1
4 days	8.5	180	70	0.8	0.47	64	0.9
5 days	8.7	130	130	0.8	0.50	49	1.0
6 days	8.8	80	80	0.7	0.52	42	1.0

\*Values represent the average of both media.

mycin and the physical consistency of the mycelia were not the same in the two media. The over-all destruction of streptomycin was rapid, either it was metabolized or inactivated at the alkaline reaction. A close relationship between the hydrogen ion concentration and the concentration of ammonia was again evident, apparently they are directly related. The  $\alpha$ -amino nitrogen dropped to a minimum; apparently as proteolysis proceeded the free

amino acids were immediately utilized as shown by the accumulation of ammonia. In the sodium chloride deficient medium, lysis of the mycelium was accompanied by an increase in streptomycin in the medium, until equal to the sodium chloride medium.

This observation provided an explanation of the action of sodium chloride. As the concentration of salt increases in the medium the permeability of the membrane is affected in such a way as to cause an accumulation of streptomycin within the mycelium that is only liberated by lysis of the cell. In order to prove this hypothesis, mycelium was produced on Waksman's medium with and without sodium chloride and 10-ml. aliquots were transferred to centrifuge tubes. The medium was separated from the mycelium by centrifugation and the mycelium thoroughly washed by dispersing in water and recentrifuging. The mycelium of both media was treated as follows (Table VI).

TABLE VI

THE EFFECT OF SODIUM CHLORIDE IN WAKSMAN'S MEDIUM IN THE DISTRIBUTION OF STREPTOMYCIN BETWEEN MYCELIUM AND MEDIUM

Treatment of mycelium	Mycelium from medium without NaCl	Mycelium from medium with NaCl
	Zone of inhibition in mm.	
Tap water added, stored in refrig. (7 days)	27.0	21.0
0.5% NaCl added, stored in refrig. (7 days)	25.5	22.0
1% NaCl added, stored in refrig. (7 days)	26.0	22.0
0.1M phosphate, pH 7 added, stored in refrig. (7 days)	25.5	22.5
0.2M phosphate, pH 7 added, stored in refrig. (7 days)	26.5	22.0
Shaken with tap water 24 hr.	23.5	21.0
Shaken with 0.5% NaCl 24 hr.	26.0	23.5
Ground in mortar	26.5	22.5
Ground in mortar and mixture suspended in 0.1M phosphate buffer pH 7; stored in cold (7 days)	25.5	22.0

All volumes were kept constant and the results are comparable. Excellent agreement in the amount of streptomycin produced was obtained by mechanical rupture of the mycelium, as compared to shaking in saline or slow diffusion at 4° C. for seven days. From these results it seems evident that streptomycin synthesis does not occur when the metabolic activity is at a minimum.

An exact study of the daily variations in the distribution of streptomycin between mycelium and media was next carried out (Table VII). There appears to be a limit to the amount of streptomycin synthesized because the total production is constant; however, the distribution varies with salt concentration and duration of fermentation. As lysis proceeds, the streptomycin is liberated from the mycelium and is destroyed at the same rate in both media. The antibiotic does not seem to be metabolized but is most probably inactivated by the accumulation of alkali.

TABLE VII

STREPTOMYCIN DISTRIBUTION BETWEEN MYCELIUM AND MEDIA—WAKSMAN'S MEDIUM  
WITH AND WITHOUT SODIUM CHLORIDE

Days	Medium with NaCl		Medium without NaCl	
	Zone of inhibition (mm.)			
	Mycelium	Medium	Mycelium	Medium
1	11.0	23.0	21.0	23.5
2	23.0	25.5	26.0	21.0
3	22.0	26.5	25.0	22.5
4	20.0	25.5	20.0	25.0
5	19.0	24.5	19.0	24.5

Since it has been reported (7) that some ions can replace sodium chloride, whereas others can not, an experiment was carried out in which the sodium or chloride ion was replaced by other cations or anions. Little or no difference was observed in the cation series. With the anions a more selective action was observed. This was apparent, not only in the distribution of streptomycin but also in the type of mycelium produced. With tartrate the mycelium was dense and dark in color, while with the thiocyanate it appeared fluffy, swollen, and light in color. This was proof that the action of the sodium or chloride ion was not specific but that the anion and cation could be replaced by others, provided they were close to the sodium or chloride ion in the Hofmeister lyotropic series.

These observations suggest the possibility of developing new methods for the isolation of streptomycin. Rather than recovering the antibiotic from a deeply colored, highly complex medium, it might be easier to isolate it from the mycelium. As Molitor (6) pointed out, "fundamental changes will have to be brought about in the present isolation and purification technique in order to obtain streptomycin concentrates not contaminated with biologically active substances."

### Summary and Conclusion

Amongst the amino acids, histidine alone serves as a readily available nitrogen source that promotes both streptomycin and mycelium production. Valine, however, plays a more specific role, it promotes streptomycin synthesis only, provided other factors are present that will promote the formation of mycelium. The addition of aspartic acid definitely inhibits synthesis of the antibiotic but accelerates production of mycelium and induces similar metabolic changes in the medium as does a complex organic medium. Since aspartic, glutamic, or succinic acid exhibit a similar effect upon the organism, it may be concluded that the synthesis of mycelium proceeds by the aspartase-transaminase system but this inhibits streptomycin formation. However, another mechanism, as yet unexplained, will also bring about the synthesis of mycelial

protein and apparently there is some connection between this alternate path of mycelial synthesis, the role of valine, the action of inositol, and the production of streptomycin.

The influence of high concentrations of sodium chloride can not be ascribed to osmotic pressure effects. It appears more likely, in view of the observations with the ions of the lyotropic series, that the permeability of the membrane is affected by the concentration as well as the nature of the ion. These observations also suggest that the antibiotic is a product of intracellular synthesis and diffuses into the medium when the salt concentration is high. Unexplained, however, is the observation that on the second day of fermentation in a medium without added sodium chloride, more streptomycin is found in the medium than in the mycelium.

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## SOME FACTORS INFLUENCING THE GROWTH AND SURVIVAL OF RHIZOBIA IN HUMUS AND SOIL CULTURES

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### Abstract

A study was made of the growth of rhizobia outside the host plant in three high humus mixtures and a mineral soil. Rhizobia grew better in pure cultures than in an unsterile base. Incubation in Erlenmeyer flasks gave, as a rule, higher counts than in paper cartons. Addition of a mixture of sucrose, calcium carbonate, sodium chloride, dipotassium phosphate, and magnesium sulphate led to increased numbers of rhizobia in pure culture in all bases used, and produced increased numbers in the unsterilized mineral soil also. Addition of this mixture to the unsterile high humus bases increased the total numbers of bacteria, but not the number of rhizobia. When the bases were sterilized, the highest counts of rhizobia were obtained in the high humus bases. When sterilization was not carried out, the greatest numbers of rhizobia occurred in the mineral soil. In tightly stoppered flasks that did not permit aeration, the rhizobia appeared to die out completely when stored for more than a few days, whereas in loosely stoppered flasks the counts remained at a high level until the base became desiccated.

### Introduction

To obtain the optimum growth of leguminous crops, inoculation of the seed with legume bacteria is frequently necessary. When inoculating legume seed, there are two important factors that must be considered. First the inoculant applied to the seed should contain large numbers of bacteria of the cross-inoculation group in question, and second, these bacteria should be a strain that is efficient in fixing nitrogen in association with the inoculated legume. It was with the first of these problems in mind that this study was initiated. The effects of aeration, moisture content, and addition of nutrient salts on the growth of rhizobia in humus and soil cultures were investigated.

### Review of Literature

The first use of soil as a base in which to grow legume bacteria was reported by Simon (22). Since moist soil cultures were first introduced, their use has increased widely. Temple (24) also has reported on steam sterilized soils as a medium for growing rhizobia. He compared the numbers of rhizobia per gram of soil with the numbers in a nutrient solution and found the latter very inferior as a medium in which to grow the organisms. Similar results were obtained using legume bacteria from crimson clover and alfalfa. The soil used by Temple was low in fertility, and he found that by the addition of various organic compounds such as alfalfa meal, sucrose, etc., he was able to increase the numbers of bacteria very markedly. In this work, however, counts were not continued beyond the 42nd day.

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1936 Hofer (12) made a study of the numbers of rhizobia in humus. He reported that the numbers were highly variable. After one the count was usually higher than immediately after adding culture mus base, but after 50 days it was lower. After one year the count dule bacteria was only 1% of what it had been immediately after ation.

ers (5) carried out extensive experiments on commercial cultures of e inoculants. In his work he studied the numbers of bacteria in the s inoculants as well as their ability to cause infection of the host plant. ound that the numbers of bacteria varied greatly. In general those es with less than one million organisms per gram were not effective, gh it was impossible to set definite limits. He found that cultures that gh plate counts usually gave satisfactory nodulation of legume plants. e importance of free access to air on the growth and survival of rhizobia en emphasized by a number of workers including Alicante (1), Gangulee Fred *et al.* (8), and Ockerblad (18). There are, however, reports ards (4) and Jones (13) ) that indicate that the rhizobia are able to e for many years when stored in sealed containers.

(20) noted the beneficial effect of small amounts of calcium sulphate e growth of pure cultures of rhizobia. Truesdell (26) observed that a amount of added phosphorus increased the number of alfalfa nodule ia in sterilized soils. Fulmer (9) reported that the addition of calcium ate or magnesium carbonate greatly stimulated the growth of rhizobia alfalfa or lupines in sterilized acid soil. Thornton (25) and Alicante (1) press the importance of calcium on the growth of legume bacteria outside st plant.

e all nonsporeforming bacteria, the rhizobia are sensitive to excessive . The effect of desiccation, however, depends on several conditions— ture of the substrate, reaction, temperature, etc. Giltner and Lang- y (11) carried out extensive studies on the longevity of rhizobia under s moisture conditions. They found that rhizobia survive a long time dry soil, probably because of the films of hygroscopic moisture that surround the particles. Vandecaveye (27) and Temple (24) have likewise reported on the remarkable viability of rhizobia in air-dried soil.

Another factor that appears to influence the growth of rhizobia in soil is the symbiotic or antagonistic effect of other microorganisms. As early as 1899, Nobbe and Hiltner (17) suggested that the competition between rhizobia and the rest of the soil flora results in harm to the rhizobia. They implicated both bacteria and fungi in the opposition. Sackett (21), in a laboratory study of associated growth of *B. ramosus* and rhizobia, observed that multi- plication of the latter is greatly inhibited. Duggar and Prucha (3) obtained better growth of rhizobia in sterilized than in unsterilized soil, and argued that the general soil flora have an antagonistic action towards rhizobia.



At Wisconsin Konishi (14) obtained some interesting data on the effect of certain soil bacteria on rhizobia. He isolated several unidentified organisms from the soil and tested them with rhizobia in a liquid medium, with and without calcium carbonate. In general, antagonistic effects of other microorganisms towards rhizobia were most severe in liquid media without carbonate and least severe in soil.

There are also reports of beneficial effects of certain organisms upon rhizobia. Bottomley (2) reported enhanced nitrogen fixation by mixed cultures of bacteria and *Azotobacter*. Manns and Goheen (16) and Fellers (5) referred to a beneficial effect from the association of *Azotobacter* and rhizobia upon formation in Petri plates. Löhnis and Hansen (15) also noted the stimulatory effect of *B. radiobacter* upon colony formation of the cowpea-soybean inoculation group.

### Outline of Investigation

In this work four different inoculant bases or media were used for culturing the rhizobia.

These materials were:

1. Winterburn peat. This is a high lime peat obtained in a bog at Winterburn, which is several miles west of Edmonton. The peat used was fairly decomposed material obtained at a depth of approximately four feet.

2. Peat - black soil mixture. This base was prepared by mixing equal portions by weight of the base described above with a black mineral soil from the University plots just south of Edmonton.

3. Peat - carbon black mixture. This inoculant base consisted of well-decomposed peat obtained near Edmonton. The peat was mixed with a small amount of carbon black. This material was obtained through the courtesy of Mr. J. A. Robertson of Edmonton, who had used it as an inoculant base in preparing commercial cultures of rhizobia.

4. Edmonton loam. This is a black zone soil from the University plots just south of the city of Edmonton. This soil comprised one-half of inoculant number two.

Cultures of *R. trifolii* and *R. leguminosarum* were stored in the four media listed above. Containers holding the various bases were inoculated with cultures of these two organisms and were stored at room temperature under a variety of conditions. Plate counts of the bacteria were made at intervals during the storage period—usually at 10, 40, 70, and 140 days after inoculation.

The plan of the experiments is shown in Table I. As indicated in Table I, storage was carried out in paper cartons for two of the experiments, IV and VII. In the other experiments storage was carried out in Erlenmeyer flasks. Where cartons were used, they were waxed over at the beginning of the storage period to prevent too rapid a loss of moisture. When storage was carried out in Erlenmeyer flasks, some of the flasks were tightly stoppered, whereas others were stoppered loosely so that a free exchange of air was possible. To stopper

TABLE I

PLAN OF EXPERIMENTS ON THE GROWTH AND SURVIVAL OF *R. leguminosarum* (EXPTS. I TO IV, TABLES II TO V) AND *R. trifolii* (EXPTS. V TO VII) IN HUMUS AND SOIL CULTURES

	Bases used	Stopperings	Treatments		Storage periods, days
Expt. I	Peat	Tightly stoppered	Sterile and unsterile	No nutrients added	0
	Peat - black soil	Cotton stoppered			10
	Peat-carbon mixture				38
	Black soil				72
					148
Expt. II	Same as above	All cotton stoppered	All sterile	Nutrients added to half of flasks	0
					14
					42
					90
					141
Expt. III	Peat-carbon mixture Black soil	All cotton stoppered	All unsterile	Nutrients added to half of flasks	0
					12
					25
					70
					112
Expt. IV	Peat Peat - black soil Peat-carbon mixture Black soil	Waxed paper cartons	Sterile and unsterile	No nutrients added	0
					11
					48
					77
					146
Expt. V	Same as Expt. I except <i>R. trifolii</i> used				0
					14
					38
					70
					142
Expt. VI	Same as Expt. II except <i>R. trifolii</i> used				0
					20
					48
					89
					140
Expt. VII	Same as Expt. III except <i>R. trifolii</i> used				0
					8
					36
					68
					146

flasks tightly, rubber stoppers waxed over with paraffin were used. In stoppering flasks loosely, on the other hand, the practice was to use a rubber stopper one size smaller than would normally have been used, and to wrap a little absorbent cotton around it. This allowed for a free exchange of air in the Erlenmeyer flask, but the drying out was less rapid than would have been the case had an ordinary cotton stopper been used.

In those cases where the inoculant base was supplemented by the addition of nutrients, the following mixture was used:

Sucrose	— 100 gm.
Dipotassium hydrogen phosphate	— 10 gm.
Sodium chloride	— 2 gm.
Magnesium sulphate	— 4 gm.
Calcium carbonate	— 60 gm.

These salts and sucrose were used in the proportions given above in the media recommended by Fred *et al.* (6) and by Fred and Waksman (7) for plating legume bacteria. It was added to the inoculant base at the rate of 1.4% by weight.

In a number of the experiments the growth and survival of pure cultures of rhizobia were studied. In such cases the humus bases were sterilized in the autoclave at 15 lb. steam pressure (121° C.). The peat, peat - black soil mixture, and the peat-carbon mixture were held at this temperature for two and a half hours, while the black soil was heated in the autoclave at 121° C. for 25 min. Simpson (23) found that this treatment gave complete sterilization of these bases in the amounts used here.

Inoculations were made by using a suspension of bacteria in a 0.75 % sodium chloride solution. To make up this suspension bacteria were grown on agar slants in Erlenmeyer flasks. After a good growth had been obtained the sodium chloride solution was added, the growth scraped free from the surface of the agar with a sterile inoculating needle, and a suspension formed by rotating the flask to get an even distribution of the bacteria. This suspension was added to the inoculant bases at the rate of 10 cc. per 50 gm. of base. Counts of the number of legume bacteria were made at the time of inoculation. From this, the initial count of legume bacteria per gram of base could be calculated. All counts were made by the plate method using as the medium yeast extract - sucrose agar containing Congo red, as recommended by Fred *et al.* (6) and by Fred and Waksman (7). The Congo red aids in the detection of contaminants that might be present. Detection is possible since most of the contaminants absorb the dye, thus producing reddish colonies, whereas the colonies of legume bacteria remain clear.

At the beginning of the storage periods all bases were made up to 30% of their total water holding capacities. This 30% included the water carrying the suspension of nodule bacteria. The tightly stoppered flasks remained at this water content throughout the storage period. Weighings of the flasks at the beginning and at the end of the storage period indicated that there was no moisture loss from these flasks. All water percentages given in the tables are expressed on the air-dry basis.

## Results

The results of Expts. I to IV, including bacterial counts and moisture percentages in the different inoculant bases are given in Tables II to V. All counts are in millions per gram and the incubation times and treatments are indicated in the tables. The counts and moisture percentages are on the air-dry basis.

The counts of bacteria in the inoculant bases studied in this investigation are very closely correlated with the moisture content and aeration of the inoculants. This is particularly true of the counts of rhizobia, but is also very noticeable in the total counts of unsterilized inoculant bases.

TABLE II

EFFECT OF VARIOUS TREATMENTS ON PLATE COUNTS OF *R. leguminosarum* (B<sub>1</sub>) AND OTHER MICROORGANISMS IN FOUR INOCULANTS—PEAT, PEAT—BLACK SOIL MIXTURE, PEAT—CARBON MIXTURE, AND BLACK SOIL (MILLIONS PER GRAM)

Storage time in days	Sterile base						Unsterile base					
	Stoppering											
	Rubber			Cotton			Rubber			Cotton		
	Nodule bacteria count	Total count	Water, %	Nodule bacteria count	Total count	Water, %	Nodule bacteria count	Total count	Water, %	Nodule bacteria count	Total count	Water, %

*Peat (no nutrients added)*

0	30.4	30.4	137.4	30.4	30.4	137.4	30.4	32.9	137.4	30.4	32.9	137.4
10	181.5	181.5	137.4	285.0	285.0	134.2	16.0	1011.0	137.4	15.0	819.0	135.0
38	0.0	0.0	137.4	129.0	129.0	127.4	0.0	224.0	137.4	12.0	1207.0	124.0
72	0.0	0.0	137.4	31.7	31.7	117.0	0.0	203.0	137.4	5.0	589.0	117.4
148	0.0	0.0	137.4	10.6	10.6	101.8	0.0	148.0	137.4	0.0	449.0	107.4

*Peat—black soil mixture (no nutrients added)*

0	30.4	30.4	81.0	30.4	30.4	81.0	30.4	31.9	81.0	30.4	31.9	81.0
10	292.0	292.0	81.0	341.0	341.0	78.8	11.5	275.0	81.0	3.3	416.5	78.8
38	0.2	0.2	81.0	190.0	190.0	72.4	0.0	205.0	81.0	7.0	261.0	71.0
72	0.0	0.0	81.0	238.0	238.0	57.6	4.0	116.0	81.0	5.5	195.0	63.2
148	0.0	0.0	81.0	50.0	50.0	42.0	0.0	158.0	81.0	0.0	131.0	50.2

*Peat—carbon mixture (no nutrients added)*

0	30.4	30.4	69.2	30.4	30.4	69.2	30.4	100.2	69.2	30.4	100.2	69.2
10	490.0	490.0	69.2	570.0	570.0	67.2	20.0	396.5	69.2	8.8	784.0	67.4
38	0.0	0.0	69.2	160.0	160.0	60.8	0.0	101.0	69.2	9.0	921.2	62.6
72	0.0	0.0	69.2	376.0	376.0	50.8	0.0	193.0	69.2	0.8	617.0	53.2
148	0.0	0.0	69.2	62.0	62.0	27.8	0.0	507.0	69.2	0.0	401.2	34.2

*Black soil (no nutrients added)*

0	30.4	30.4	29.2	30.4	30.4	29.2	30.4	30.9	29.2	30.4	30.9	29.2
10	265.0	265.0	29.2	303.0	303.0	27.6	58.0	58.0	29.2	50.0	55.0	28.0
38	60.0	60.0	29.2	258.0	258.0	20.2	98.0	183.0	29.2	287.0	454.0	22.0
72	0.0	0.0	29.2	142.0	142.0	16.6	3.3	10.6	29.2	1.6	6.4	15.0
148	0.0	0.0	29.2	0.0	0.0	5.0	2.9	11.2	29.2	0.0	59.0	6.6

The results of Expt. I in Table II show very definitely that in all four inoculant bases used, the numbers of rhizobia drop sharply when stored in sealed flasks. This is particularly noticeable in the peat, peat—black soil, and peat—carbon mixtures. In these tightly stoppered sterile bases the numbers of rhizobia rose for a time, being considerably higher when the second plate count was made after storage for 10 days than they had been at the time

of inoculation. In the third and subsequent counts in these three bases, the numbers had dropped below 50 thousand per gram.

In the fourth base, black soil, at 38 days the count of rhizobia in tightly stoppered flasks was still higher than at the beginning of the storage period. However, in this base the numbers of rhizobia had dropped below 50 thousand per gram by the time the fourth count was made 72 days after the beginning of the storage period.

In the unsterile, tightly stoppered flasks, results somewhat similar to those with sterile bases were obtained. In the peat, peat - black soil, and peat-carbon mixture, when the third count was made after storage for 38 days, no typical colonies of rhizobia appeared on the plates. There was a slight discrepancy in the peat - black soil mixture where a count of four million rhizobia per gram was obtained in the fourth count at 72 days, but this had again dropped below 50 thousand per gram when the fifth count was made. An interesting fact brought out here is that, unlike the counts in the sterile containers, when the second count was made 10 days after the beginning of the storage period, the count of rhizobia had dropped far below the initial numbers. This would seem to indicate that the report made by Fellers (5) that it is unnecessary to sterilize peat or soil when used as a base for culturing rhizobia is incorrect. The presence of other soil organisms seems to exert a very strong antagonistic effect upon the growth of rhizobia. This is indicated by the fact that there was a high total count coupled with these low counts of rhizobia.

In the unsterile, tightly stoppered flasks the total count rose quickly from the beginning of the storage period until the second count was made at 10 days, and then fell quite sharply in the succeeding counts. In the peat - black soil mixture and in the peat-carbon mixture the plate counts indicated a rise in the total numbers of bacteria between the fourth and fifth counts. This may have been due to errors in the plate counts or merely to a variation in the different flasks. In the unsterile tightly stoppered flasks containing black soil, the total count of bacteria did not rise as rapidly as in the other three inoculants. However, the count of rhizobia did rise quite markedly in this base, reaching 98 million per gram after 38 days' storage. The number of rhizobia as well as the total number of bacteria had dropped quite sharply in this base by the time the fourth and fifth counts were made.

In the cotton stoppered, sterile flasks, the counts in all bases had risen far above the number initially introduced when the second count was made. A marked drop was evident when the third count was made after 38 days' storage. A slight rise took place in the peat-carbon and peat - black soil mixtures between the third and fourth counts, while in the peat and in the black soil the numbers of rhizobia continued to drop. When the fifth count was made after 148 days' storage, no rhizobia were present in the black soil, while the peat-carbon mixture had the highest count, 62 millions per gram.

In the unsterile, cotton stoppered flasks as in the unsterile, tightly stoppered flasks, the numbers of rhizobia had dropped sharply in all bases except the

black soil, when the second count was made. The number of rhizobia in the peat - black soil mixture rose slightly between the second and third counts but fell again thereafter. In all of these bases no rhizobia were present when the final counts were made after 148 days' storage. Once again, as in the tightly stoppered flasks, counts of rhizobia and other bacteria in the black soil were higher after 10 days' storage than they had been initially. The number of rhizobia had risen still higher after 38 days' storage. Numbers of rhizobia dropped quite sharply between the third and fourth counts and when the fifth count was made after 148 days' storage none were found.

The total counts in all four bases in unsterile cotton stoppered flasks showed, in general, a sharp rise, followed by a fall in numbers. In peat, peat-carbon, and black soil the largest numbers of bacteria were counted after 38 days' storage, while in the peat - black soil mixture the largest number was counted after 10 days' storage.

The counts of rhizobia given in Table III (Expt. II) show that where nutrients are added to the inoculant bases there is a marked increase in the numbers of nodule bacteria. The only exceptions to this are in the fourth counts of the numbers of rhizobia in the peat and the peat - black soil mixture. Here the number of rhizobia in the base to which no nutrients were added was higher than where nutrients were added. As in Table II, the results indicate the importance of moisture content on the numbers of rhizobia. The numbers rose for a time, but as the percentage of moisture in the base fell, the numbers of bacteria dropped quite markedly as well. The greatest numbers were usually present at the second count (14 days) but sometimes at the third (42 days). The most marked increases in numbers of legume bacteria resulting from the addition of nutrients were obtained in the peat - black soil and peat-carbon mixtures. With no nutrients added, the counts of rhizobia in the peat-carbon mixture were considerably higher than in the peat - black soil mixture. Another interesting point in the data of this table is that the counts of rhizobia in the peat-carbon mixture treated with nutrients remained very high for 90 days. In all the other treatments, the numbers had dropped to a fraction of their highest count by this time.

A comparison of the counts in flasks to which no nutrients were added, in Table III, with those similarly treated in Table II, shows that these counts follow similar trends. In both cases the numbers increased for a time and then dropped sharply as the bases dried out.

Table IV gives the results of Expt. III. It was thought that it might be more practical, if possible, to use unsterilized inoculant bases. Therefore, in this experiment a study was made of the growth of rhizobia in unsterile inoculant bases with and without added nutrients.

In the peat-carbon mixture there seems to be no significant difference between counts of rhizobia in the base to which nutrients were and were not added. There was, however, a definite increase in the total number of bacteria

TABLE III

PLATE COUNTS OF *R. leguminosarum* (B<sub>8</sub>) IN THE FOUR INOCULANTS STORED IN ERLLENMEYER FLASKS, STERILIZED AND STOPPERED WITH COTTON (MILLIONS PER GRAM)

Storage time in days	Nutrients added		No nutrients added	
	Nodule bacteria count	Water, %	Nodule bacteria count	Water, %
<i>Peat</i>				
0	63.3	137.4	63.3	137.4
14	646.0	134.0	286.0	134.2
42	138.0	125.2	114.0	127.6
90	44.0	110.6	53.0	113.8
141	28.7	95.6	10.5	89.6
<i>Peat - black soil mixture</i>				
0	63.3	81.0	63.3	81.0
14	1794.0	77.0	526.0	78.6
42	1585.0	73.6	350.0	71.0
90	208.0	57.4	216.0	53.2
141	97.5	46.6	40.9	39.0
<i>Peat - carbon mixture</i>				
0	63.3	69.2	63.3	69.2
14	1997.0	65.4	798.0	66.4
42	1617.5	59.2	918.0	56.6
90	1678.0	40.0	364.0	40.2
141	291.0	31.4	176.0	23.8
<i>Black soil</i>				
0	63.3	29.2	63.3	29.2
14	578.0	24.4	337.5	25.0
42	676.2	20.8	352.0	19.0
90	26.0	3.8	4.9	4.2
141	3.1	0.8	1.3	0.0

in the base to which nutrients were added. This suggests that the competition of additional soil bacteria must have offset the beneficial effect derived by rhizobia from the nutrients added. In the black soil the addition of nutrients gave a very definite increase in numbers of rhizobia as well as in total count, over the base to which no nutrients had been added. In the soil to which no nutrients were added, the numbers of rhizobia did not increase above the initial number added. Where nutrients were added, after 12 days' incubation, the number of rhizobia had risen to approximately double the initial count. This is in marked contrast with the counts of rhizobia in the

TABLE IV

PLATE COUNTS OF *R. leguminosarum* (B<sub>1</sub>) IN TWO INOCULANTS, STORED IN ERLENMEYER FLASKS, UNSTERILE AND STOPPERED WITH COTTON (MILLIONS PER GRAM)

Storage time in days	Nutrients added			No nutrients added		
	Nodule bacteria count	Total count	Water, %	Nodule bacteria count	Total count	Water, %
<i>Peat-carbon mixture</i>						
0	101.2	181.0	69.2	101.2	181.0	69.2
12	15.0	1189.0	64.4	32.0	741.0	64.2
25	11.0	1021.0	62.0	9.0	425.0	54.8
70	4.0	541.0	41.4	5.0	406.0	48.4
112	0.1	512.0	32.4	0.4	308.0	35.2
<i>Black soil</i>						
0	101.2	101.7	29.2	101.2	101.7	29.2
12	198.0	296.0	25.2	87.0	127.0	25.2
25	168.0	371.0	21.4	92.5	162.5	22.8
70	5.5	95.0	8.8	1.0	22.5	14.2
112	13.0	90.0	5.8	0.5	2.3	5.6

peat-carbon mixture, where after 12 days' incubation, the number of rhizobia present had dropped to a fraction of those originally present.

Table V represents the results of Expt. IV and contains the bacterial counts for inoculants stored in paper cartons. It includes counts for both sterile and unsterile inoculant bases. In the paper cartons containing sterilized inoculant base, some recontamination did take place. However, as the total counts were not recorded, it is impossible to say what effect this might have had on the numbers of rhizobia. In the sterilized bases the highest counts were obtained after 11 days' storage. The numbers were still fairly high after 77 days, but were quite small after 146 days, and in one case they had disappeared entirely after 146 days.

A comparison of the data in this table with that in Tables II and III indicates that the numbers of rhizobia in sterile bases stored in cartons do not rise as high as in similarly treated bases stored in Erlenmeyer flasks. The reason for the Erlenmeyer flasks being better containers in which to culture rhizobia seems hard to determine. There are several factors that may have been contributory. Although total counts were not recorded, it was noticed that in many of the sterilized bases stored in cartons appreciable mold growth had taken place on the surface of the base. The mold may have had an inhibitory effect on the growth of the rhizobia. The paper cartons, in spite of being waxed, usually retained a lower percentage of moisture at the end of the storage period than did the bases stored in cotton stoppered flasks. This is another



TABLE V

PLATE COUNTS OF *R. leguminosarum* (B<sub>5</sub>) IN FOUR INOCULANTS, STERILE AND UNSTERILE, STORED IN PAPER CARTONS (MILLIONS PER GRAM)

Storage time in days	Sterile			Unsterile		
	Nodule bacteria count	Total count	Water, %	Nodule bacteria count	Total count	Water, %
<i>Peat (no nutrients added)</i>						
0	35.8	35.8	137.4	35.8	38.3	137.4
11	134.0	—	134.2	8.0	908.0	130.4
48	46.0	—	128.6	13.0	945.0	105.0
77	72.0	—	114.0	1.0	754.0	102.6
146	4.3	—	86.6	0.0	541.0	63.2
<i>Peat-black soil mixture (no nutrients added)</i>						
0	35.8	35.8	81.0	35.8	37.3	81.0
11	210.0	—	77.8	14.0	277.5	78.6
48	107.5	—	69.0	6.0	271.0	62.6
77	103.0	—	59.4	3.0	229.0	63.4
146	0.0	—	30.6	0.0	130.0	30.2
<i>Peat-carbon mixture (no nutrients added)</i>						
0	35.8	35.8	69.2	35.8	115.6	69.2
11	349.0	—	66.8	14.0	878.0	64.6
48	284.0	—	58.6	3.8	853.8	53.4
77	199.0	—	48.8	1.0	267.0	26.8
146	11.6	—	20.4	1.0	391.0	22.0
<i>Black soil (no nutrients added)</i>						
0	35.8	35.8	29.2	35.8	36.3	29.2
11	147.0	—	27.4	3.0	13.3	25.8
48	45.0	—	22.4	0.2	10.0	16.2
77	21.5	—	18.8	0.7	7.7	15.4
146	1.0	—	9.6	0.2	8.3	9.2

factor that could have contributed to the lower count of rhizobia in the bases stored in cartons. However, it is questionable whether this could account for all the difference since in the early stages of storage there was usually little difference between the moisture contents of the bases in the cartons and those in the flasks. A third possible factor is the difference in the initial number of bacteria introduced into the bases in the various experiments. In view of the rapid multiplication of bacteria under favorable conditions, it seems questionable whether this was of importance.

In the unsterilized bases stored in paper cartons, a similar trend to that obtained in unsterilized bases stored in Erlenmeyer flasks is evident. Here

TABLE VI

STORAGE PERIODS BETWEEN WHICH *R. leguminosarum* FELL FROM OVER ONE MILLION PER GRAM TO LESS THAN ONE MILLION PER GRAM

Treatment	Peat	Peat - black soil mixture	Peat-carbon mixture	Black soil
	Days			
Sterilized flasks, no nutrients added, tightly stoppered	10 - 38	10 - 38	10 - 38	38 - 72
Sterilized flasks, no nutrients added, cotton stoppered	More than 148	More than 148	More than 148	72 - 148
Unsterilized flasks, no nutrients added, tightly stoppered	10 - 38	10 - 38	10 - 38	More than 148
Unsterilized flasks, no nutrients added, cotton stoppered	72 - 148	72 - 148	38 - 72	72 - 148
Sterilized flasks, nutrients added, cotton stoppered	More than 141	More than 141	More than 141	More than 141
Sterilized flasks, no nutrients added, cotton stoppered	More than 141	More than 141	More than 141	More than 141
Unsterilized flasks, nutrients added, cotton stoppered	—	—	70 - 112	More than 112
Unsterilized flasks, no nutrients added, cotton stoppered	—	—	70 - 112	70 - 112
Sterilized cartons, no nutrients added	More than 146	77 - 146	More than 146	146*
Unsterilized cartons, no nutrients added	77 - 146	77 - 146	146*	11 - 48

— Not determined.

\* Count of one million per gram at 146 days.

the tendency was to obtain high total counts coupled with low counts of rhizobia. As in other counts of rhizobia in unsterilized inoculant bases, the numbers of rhizobia had dropped quite sharply by the time that the second plate counts were made after about 11 days' storage. They continued to drop in subsequent plate counts. When the final counts were carried out in the peat and peat - black soil mixtures after 146 days' storage, no rhizobia were present, while in the peat - carbon black mixture and the black soil, the numbers were 1.0 and 0.2 millions per gram respectively. The total count in the unsterilized bases rose quite high except in the black soil, but dropped off gradually as the inoculant base dried out.

Experiments were also carried out using red clover bacteria instead of pea bacteria. These experiments were, in all other respects, identical with Expts. I, II, and IV reported in Tables II, III, and V. Since the results were very similar to those obtained with pea bacteria, the data have not been included.

It is realized that it would have been desirable to supplement the plate counts of rhizobia in the inoculants with cultural experiments. However, time and facilities did not permit the carrying out of such work. Nevertheless Table VI has been set up to indicate the length of time for which the various inoculant bases remained effective. This is based on results reported by Fellers (5). He found that cultures that contained more than one million rhizobia per gram usually were effective in producing nodules on legume plants when applied to the seed before planting. Such a criterion of effectiveness of cultures is, however, merely a guide.

The data indicate, on the basis of this criterion, that all of the tightly stoppered sterilized and unsterilized inoculants except black soil remained effective for less than 38 days, black soil inoculants remaining effective for somewhat longer periods. In the sterilized cotton stoppered flasks all of the inoculants except black soil remained effective for more than 148 days, black soil remaining effective for more than 72 but less than 148 days. In another experiment all of the previously sterilized cotton stoppered flask inoculants, with and without the addition of nutrients, remained effective for more than 141 days. In the unsterilized cotton stoppered flasks all of the inoculants remained effective for more than 72 days, but less than 148 days, except the peat-carbon mixture, which remained effective for more than 38 days but less than 72.

In addition to counts of bacteria, the inoculant bases were analyzed for available phosphorus, potassium, calcium, nitrate nitrogen, and ammoniacal nitrogen. This was done by using the method of Peech and English (19). It was hoped from this work that it would be possible to correlate the amounts of the above-mentioned ions present with the growth of rhizobia in the bases. However, the method of analysis was not always sensitive enough to reflect the small differences in chemical composition brought about by differences in treatment. It did indicate that sterilization of high humus bases increased the content of ammoniacal nitrogen very markedly. Addition of the nutrient mixture containing sucrose, calcium carbonate, dipotassium phosphate, sodium chloride, and magnesium sulphate was usually reflected by increased amounts of potassium and phosphorus as shown by this method. Additions of calcium were not usually reflected by this method of analysis. Similarly, treatment differences such as sterilization, length of incubation, etc. did not show any consistent difference in chemical composition as determined by this method. Thus it was impossible to make the desired correlation between availability of these ions and the growth of the rhizobia.

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## FLUORESCENCE MICROSCOPE EXAMINATION OF BACTERIA IN SOIL<sup>1</sup>

BY S. STRUGGER<sup>2</sup>

### Abstract

By the staining of soil with the fluorescent dye acridinorange and examination of the stained soil under the fluorescence microscope, it is possible to observe directly the living bacteria of soil in their autochthonic condition. All humus substances are stained red in color. The bacteria, living on the humus particles, are of green fluorescence and give an excellent contrast against the red background. The bacteria are vitally stained and can be used for culture experiments. Using this method in some soil tests the autochthonic bacteria of soil have been investigated qualitatively and quantitatively.

In microbiological research on soil, two methods may be used.

1. With the aid of culture plates the organisms are isolated from the soil and identified. In general, all microscopical examinations of the soil have been made by this method (on which the hitherto existing results of microbiological results are therefore based) but they do not permit any conclusion about the autochthonic condition of the soil bacteria. Quantitative determination of the bacteria, too, has been performed in this way. The results do not stand critical examination, however, because not all soil microorganisms can grow on the culture media used or can produce countable colonies.

2. Conn (1918 (1), 1929 (2)) and Winogradsky (1925 (6, 7), 1928 (8)) tried to observe the bacteria of soil by direct microscopical examination of stained smears. Until now, however, this method of direct microscopical analysis has not been very successful because, with the stain ordinarily used, the bacteria and the particles of soil are stained the same color. Therefore a distinction between the particles of soil and the bacteria is possible only under the most favorable conditions. There is no doubt that the direct microscopical examination of the autochthonic soil bacteria is a desirable objective of the microscopical research of soil that could not be reached until now because of technical difficulties.

In this paper will be given information about a new method of vital staining of soil bacteria with the fluorescent dye, acridinorange; this permits an analysis of the autochthonic bacteria of soil by means of the fluorescence microscope.

### General Remarks about the Acridinorange Method

In 1940, Strugger (3) succeeded in solving the problem of the vital staining of cytoplasm and nucleus of plant cells with the aid of the fluorescent dye,

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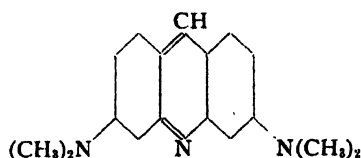
acridinorange (3,6-tetramethyldiaminoacridin).<sup>\*</sup> The albuminoid components and nucleoprotein of the living protoplasm have an extraordinary affinity for this dye. As the fluorescence microscope method of observation lies, in sensitivity, between perception by smelling and electrospectral detection of radioactivity, only an extremely small absorption of the dye in protoplasm is necessary. Therefore the harmful effect of the vital staining is so restricted that the vitally stained protoplasm of plant cells can continue to grow.

The fluorescent dye, acridinorange, is a basic dye. It is dissociated in the whole of the acid, the neutral, and the basic region up to pH 8.5.

The acridinorange cations show a variable fluorescence according to the concentration. Dissociated solutions of lower concentration (1 : 100,000 to 1 : 10,000) have green fluorescence, whereas concentrated solutions (1 : 100) shine in copper-colored fluorescence under the quartz lamp.<sup>\*\*</sup> The center of gravity of the fluorescence spectrum of weak aqueous solutions of acridinorange is displaced, reversibly, with rising concentration, to the region of long waves. This 'effect of concentration', investigated by Strugger (1940 (3)), is of great importance in the use of acridinorange as a vital stain.

If living plant cells are vitally stained by acridinorange (solution 1 : 10,000, pH 7.5) the living cytoplasm and the nucleus show green fluorescence. Acridinorange is absorbed electrostatically by the albuminoid components of the living protoplasm. But, in the living protoplasm, absorption is limited so that even after being stained with much excess dye, the protoplasm absorbs the acridinorange in low concentration only. Consequently the result is a green fluorescence of the living protoplasm. If, however, dead cells are stained with the same solution of acridinorange the dead protoplasm will develop a bright copper-colored fluorescence after a short time. The albuminoid components of dead protoplasm are able to absorb the acridinorange cations in such high concentration that a strong accumulation of the acridinorange cations, caused by electroabsorption, will result. Consequently the dead protoplasm shows a red fluorescence.

By a consideration of the position of the isoelectrical points of the different parts of the cell, the acridinorange method may be elaborated to a technique that makes possible an exact distinction between living and dead protoplasm. Strugger (4) and Strugger and Hilbrich (5) elaborated this method for the vital staining of microorganisms and for the distinction, by fluorescence microscope examination, of living and dead bacterial cells. It has been



Acridinorange

Chloride salt of 3,6-tetramethyldiaminoacridin

Manufactured by I. G. Farbenindustrie, standardized Dye Bayer.

<sup>\*\*</sup> An analysis lamp manufactured by Quarzlampengesellschaft Hanau.

shown that the protoplasm of yeast cells and all forms of bacteria can be vitally stained with acridinorange. This vital staining is so harmless that culture and animal experiments can be performed, with optimum results, with bacteria so stained. As the vital staining of bacteria with acridinorange allows a very clear observation of the cell walls and protoplasm, this method by which the stain is so simply and easily applied, is superior from every point of view to the methods of staining smears used in bacteriology until now.

### **The Method of Staining Suspensions of Soil Bacteria with Acridinorange**

For the fluorescence microscope observation of the autochthonic bacteria of soil, I thought it was theoretically important to make tests with acridinorange. The first provisional tests gave excellent results.

When a suspension of soil is stained for 10 min. with a 1 : 2000 solution of acridinorange, made with tap water, the soil absorbs most of the dissolved dye. The humus ingredients have an electronegative charge and are able to absorb the acridinorange cations very strongly. Therefore all humus particles and all particles of soil covered with humus shine in copper-colored fluorescence under the fluorescence microscope. The bacteria, however, living on the particles and layers of humus, allow an intravital absorption of acridinorange in small concentration only, and therefore they have a bright green fluorescence. Thus, it is possible to observe the green fluorescent living bacteria in excellent contrast against the red background of the stained soil.

The staining of the soil was carried out as follows. In each of five test tubes was placed 1 gm. of sifted soil. To each tube was then added 10 cc. of acridinorange solution, made with tap or spring water, and each was strongly shaken. As the different kinds of soil may differ in their ability to absorb the stain, the same kind of soil must be stained with five different concentrations of acridinorange (1 : 1000, 1 : 2000, 1 : 3000, 1 : 4000, 1 : 5000). In this way the concentration of acridinorange most suitable for the staining of the soil may be ascertained empirically. If the concentration is too low, the acridinorange solution will be totally discolored within a few minutes after being shaken, and the humus ingredients of the soil are not stained red enough. If the concentration is too high for the soil concerned, too much excess stain remains in the solution, hindering observation. When only a little excess stain remains in the solution after heavy shaking, the concentration is satisfactory. Consequently the most suitable acridinorange concentration must depend on the proportion of humus in the soil. Provided that the humus particles are fully saturated with acridinorange, the fluorescence microscope observation of the bacteria living on the soil is possible.

The staining of the soil is completed very quickly. Generally a few minutes is sufficient but it is advisable to wait 5 or 10 min. before making the fluorescence microscope observation.

Two methods may be used for the preparation of the slides.

1. For the qualitative analysis of the autochthonic bacteria of the soil the stained soil suspension must first be strongly centrifuged. The solution remaining on top is decanted and a small quantity of the soil sediment is well mixed in a drop of paraffin oil on a slide. Thus many soil particles are introduced into the paraffin and, after covering the mount with a coverslip, a most favorable observation with the fluorescence microscope is possible.

2. For counts a small drop is taken with a needle, from the stained and shaken suspension of soil, put on a slide, and covered with a coverslip. Usually the small amount of excess dye present does not interfere with the observation of the bacteria under the fluorescence microscope.

The examination of these preparations has to be carried out with a luminous blue light fluorescence microscope.\* As the bacteria are extremely small, an oil immersion lens and a high magnification have to be used. As immersion fluid the scarcely fluorescent paraffin oil is very suitable.

In the preparation of slides for counts, a counter of  $20\mu$  depth combined with a counting ocular is used. Taking into account the volume of the stained solution and the weight of the soil, a quantitative analysis of the results is possible.

If a preparation is made correctly the particles of soil covered with humus and the particles of humus shine in dim red fluorescence and the bacteria living on the particles of soil are green. When the bacteria of soil are killed by certain treatments before staining, no green colored bacteria are to be seen on the red fluorescent particles of soil.

The vitally stained bacteria of soil can be used for further cultivation. For this purpose, the smallest particles of soil are isolated and grown in a microculture in a drop of nutritive solution under microscopical control. Within a day, numerous bacterial cells develop from the cells stained green. They differ considerably in size and form from the autochthonic bacteria. By these experiments definite proof can be given that the small rod-like and sphere-shaped forms, shining green after being stained with acridinorange, are identical, indeed, with the autochthonic bacteria of the soil.

## Results

Most of the bacteria of soil live on the humus layers of the soil particles. The cells are either isolated or united in zoogloea-like colonies. Only a few live free in the water. The autochthonic bacteria are so small that they can be observed only with the best objectives. Their size is between  $0.3$  and  $1\mu$ . The forms found by cultivation are far larger and cannot be identified at once with the autochthonic forms.

\* The blue light fluorescence microscope is obtained by inserting blue filters (BG1, 2 mm. thick and BG3,  $1\frac{1}{2}$  mm. thick, manufactured by Schott and Gen. Jena, and 5% copper sulphate in a cuvette 2 to 3 cm. thick, or a solution of concentrated cuprammonium sulphate— $[\text{Cu}(\text{NH}_3)_4]\text{SO}_4$ —in a cuvette 2 to 5 cm. thick) in a luminous fluorescence microscope and by replacing the Euphos stop filter by an OG1 filter, 1 mm. thick, from Schott and Gen. Jena. Glass condensing lenses have been used.



By numerous counts it was ascertained that, depending on the quality of the soil, 1 gm. of soil contains 500 to 10,000 million bacteria. These figures refer to the living bacteria only. Only under unfavorable conditions were many dead bacteria found. After dryness, especially, the percentage of dead cells was found to be higher than usual.

TABLE I

Kind of soil	Depth of counter, mm.	Water content per gm. of fresh soil, %	Average no. of bacteria per field of view*	Rounded no. of bacteria per gm. of soil (fresh wt.)	Rounded no. of bacteria per gm. of soil (dry wt.)	Statistic figure for no. of zoogloea per 10 fields of view**
Garden soil, surface test	0.02	—	11.2	3,900,000,000	—	—
Garden soil, surface test	0.02	13.8	13.6	4,860,000,000	5,640,000,000	—
Garden soil, surface test	0.05	12.9	24.6	3,786,000,000	4,347,000,000	1.6
Same soil as above, dried 24 hr., at 37° C.	0.05	1.5	14.13	1,982,000,000	2,012,000,000	0.33
Same soil as above, watered 24 hr.	0.05	32.2	21.6	3,099,000,000	4,571,000,000	0.9
Best meadow soil, surface test	0.05	30.0	42.4	6,048,000,000	8,640,000,000	4.5
Sandy soil of heath, surface test	0.05	0.5	8.94	981,000,000	1,038,000,000	—

\* The counts have been performed with an apochromat objective n.A. 1.3, 120 $\times$  and a counting ocular 10 $\times$ .

\*\* The number of zoogloea observed in 10 fields of view is noted and after counting several preparations the average value is taken.

In Table I are given some results of counts of bacterial numbers. It is interesting to note the very marked effect of dryness on the life of soil bacteria. It may be seen, for example, that dryness lasting for only 24 hr. can markedly reduce the number of living bacteria in garden soil.

### Conclusion

The observations reported in this paper show that it is possible to carry out a practically useful, rapid estimation of the number of bacteria in soil by staining the soil with acridinorange and examining it under the fluorescence microscope. The number of bacteria in the soil changes at a very rapid rate. Generally the number rises with an increase in the quantity of humus. The distribution of the bacteria in the soil profile, too, has been investigated by this method. Generally it has been confirmed that the quantity of bacteria is at its maximum within the rhizosphere, while in the B-horizon, where fewer plant roots are growing, the quantity very rapidly decreases.

Using this method of observation described, it is now possible to investigate directly the influence of various methods of cultivation or manuring as well as the influence of climatic factors on the bacteria of soil. The acridinorange method is most suitable for the study of the rhizosphere.

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# A CONTRIBUTION TO THE STUDY OF DORMANCY IN SEEDS

LACTUCA SATIVA L.<sup>1</sup>

BY C. W. LEGGATT<sup>2</sup>

## Abstract

The ease with which lettuce seeds may be made dormant by placing them under germinating conditions in blue light suggested that this kind of seed would provide satisfactory material for a study of certain aspects of dormancy in seeds. Studies have been made of the behavior of lettuce seeds under varied conditions of moisture, atmosphere, color of light, and integrity of the testa. Measurements of the absolute respiration have provided curves of the drift with time of carbon dioxide output, oxygen uptake, and of respiratory quotient. Experiments with substances that might induce dormancy and light-sensitiveness similar to that induced by blue light have been made. The bearing of the results obtained upon possible metabolic changes in the seed are discussed.

The possibility that the observed effects of light upon seed germination are due to changes in the permeability of the seed coat induced by light is considered as untenable, but this does not exclude the fact that the seed coat is a restrictive agency to the free passage of oxygen, carbon dioxide, and other substances. Indeed, in any description of the probable metabolic state, this must certainly be considered. While it has not been possible to formulate a definite biochemical scheme to account for blue light effects, the experimental evidence suggests that 'carbon dioxide zymasis' may be promoted by blue light and that carbon dioxide may be one of the inhibiting factors. The blue light dormant condition is characterized by depressed respiration but without more disturbance of the equilibrium of the pre- and postglycolytic phases of respiratory metabolism than is found to be characteristic of uninhibited seeds as reported by other workers.

The results of this research suggest that further elucidation of the phenomenon of dormancy should be sought in the direction of more extended studies of the respiration of dormant and non-dormant seeds.

## Introduction

The failure of seeds to germinate promptly when provided with conditions 'ordinarily' suitable for the germination of seed of the species is a phenomenon of rather frequent occurrence. The existence and degree of dormancy are generally correlated inversely with the time elapsed since harvest; after a period of maturation, the dormancy becomes broken and the conditions of moisture, aeration, temperature, and substrate under which the seeds will then germinate may be considered as those 'ordinarily' suitable for seeds of the species.

Dormancy is a problem of great practical importance in the arts of agriculture, horticulture, and silviculture, and both on this account and on that of its intrinsic interest it has received the attention of a large number of workers. It presents itself as a problem in two ways: failure of prompt

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germination, under favorable conditions, of sound, healthy seeds may cause serious loss to the grower while, to the seed analyst, responsible for the enforcement of the law or for the issuing of reports that form the basis of sale, it is often a source of great embarrassment and may result in financial loss to the trader. From the point of view of physiology and ecology, germination and dormancy in seeds appear to form part of a group of related phenomena having to do with the processes of growth that are of the highest interest and importance.

Dormancy in seeds presents a very wide field and accordingly, in the present paper it has been necessary to confine the study to one, somewhat restricted, phase. Nevertheless, it is possible that the various kinds of dormancy that have been described may be more closely connected than some have thought, so that observations, even in a restricted phase may have a fairly wide application.

### I. Experiments with *Lactuca sativa* L. Preliminary Tests

In the preliminary experiments, confirmation was soon obtained of the inhibiting effect of blue light and the promotive effect of red light on lettuce seed germination as reported by Flint (2) and Flint and McAlister (3). Owing to lack of time with regard to the number of tests that had to be made, it was unfortunately necessary in this preliminary work to use rather small samples and, since the seed was rapidly undergoing a drift in metabolic equilibrium at the time, it was not possible to repeat experiments exactly. This drift is shown in Fig. 1 in which germination in the dark after between two and three days is plotted against the date when the test was made. It is evident that the seed was dark-sensitive up to the middle of February, after which time light was not necessary for its complete germination. The same sample on Jan. 15, 1935, germinated 95% after  $2\frac{3}{4}$  days with exposure to daylight. It was a sample of the variety Early Curled Simpson and was used throughout these preliminary tests.

The dark tests were conducted by setting the seeds on damp blotters in covered Petri dishes, wrapped in impenetrable black paper and set in a dark germinator at 20° C. Originally, dark Petri dishes had been prepared by covering them outside and inside with lead foil by means of Duco Household Cement and, when thoroughly dry, painting them all over with two coats of flat black. However, it was found that as long as the smell of the Duco cement persisted, seeds were completely inhibited from germinating. This was proved by smearing a little of the cement on the insides of undarkened dishes, which were used for tests when the cement had hardened thoroughly; such tests showed complete inhibition until the smell had gone.

The foregoing has a bearing on the following experiment (No. 4). Two hundred seeds were set to germinate at 20° C. on damp blotting paper in each of the following:—darkened Petri dishes (as above), Petri dishes wrapped in black paper, and in Petri dishes wrapped in blue cellophane.

Of the seeds remaining ungerminated after two days, 50 from each test were cut in such a way that the pericarp only was ruptured and 50 in such a way that the testa also was ruptured, the seeds having been allowed, previous

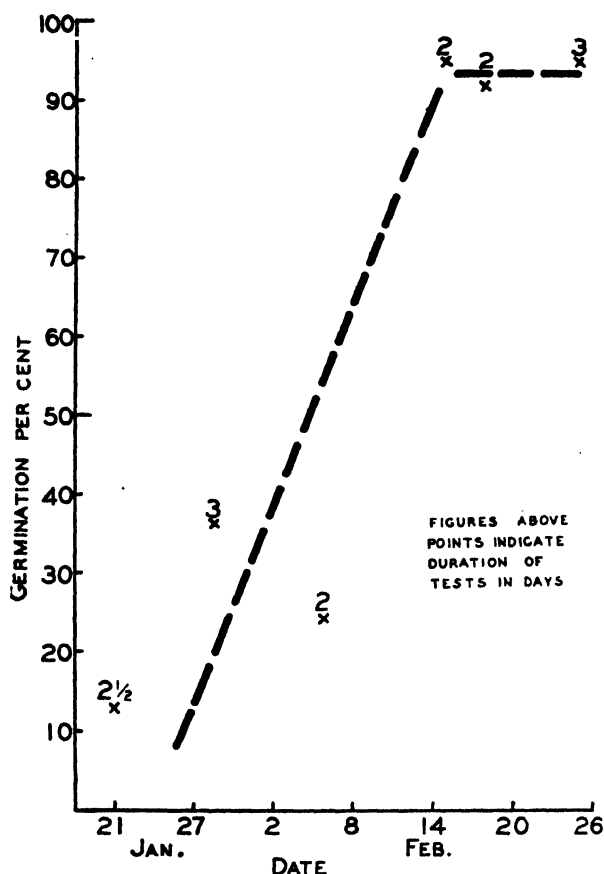


FIG. 1. Germination in dark at various dates.

to this treatment, to dry in the dark at room temperature. The seeds so treated were then set to germinate in the darkened dishes in which Duco had been used, the effects of the Duco not having been realized at that time. (Henceforward these are called the Duco dark dishes.) The germination results are given in Table I.

Four points of especial significance are to be noted:—

- (1). Where the pericarp only was ruptured, very low germination followed.
- (2). Where the testa also was ruptured, germination is practically complete in all tests after 14 days.
- (3). The 'Duco dormant' seeds were the quickest to recover while the 'dark dormant' and the 'blue light' dormant seeds lagged behind, the latter most of all.

TABLE I  
EFFECT OF RUPTURE OF PERICARP AND OF TESTA ON DORMANT SEEDS

Pretreatment	Germ., % two days	Clipped	Germination % after:						
			Days						
			2	4	6	8½	10½	14	16
'Duco dark'	0	Pericarp	0	0	0	0	2	4	4
		Testa	72	92	96	Remainder dead			
Black paper dark	24.5	Pericarp	6	6	6	6	8	10	10
		Testa	18	52	62	70	72	90	90
Blue light	0.5	Pericarp	0	0	0	0	0	Thrown out in error	
		Testa	12	34	50	60	70		

NOTE: During each count the seeds were subjected to a brief exposure to light.

- (4). The Duco dark dishes did not produce inhibition when the testa was ruptured.

It was found later that slight differences in moisture had an appreciable effect on germination, so that no significance can be definitely assigned to differences between the 'black paper dark' and 'blue light' lots. However, it is believed that this source of error in the present case is very slight since Petri dishes of the same size were used, the blotters were uniform, cut to the same size, and dipped and drained for the same time when the seeds were planted.

Expt. No. 6 was in part a repetition of Expt. 4, but Duco dark dishes were not used and the intact seeds (neither testa nor pericarp ruptured) were set to germinate in light, while the seeds with testa ruptured were set to germinate in blotters, wrapped in black paper and placed in flat tins. The date of this experiment was Feb. 16, 1935, at which time the seed had advanced rather far in its metabolic drift. The intact seeds were set to germinate, half in 'clear' light, i.e. with the Petri dishes wrapped in clear cellophane, and half in red light, the dishes being wrapped in red cellophane. Two hundred seeds were originally set in the dark and in blue light and of the ungerminated seeds there were sufficient for 30 each in red and clear light from the dark lot and 50 each in red and clear from the blue light lot, while in both lots there were 50 seeds with testa clipped. The results are presented in Table II.

In this experiment the clipped seeds have again shown much faster germination than the unclipped, but complete germination has been secured in 10 days, of intact seeds in light, as compared with their very low germination in the dark in Expt. 4. In both cases the 'clear' light has promoted germination

TABLE II  
DORMANT SEEDS INTACT AND WITH CLIPPED TESTA

Pretreatment	Germ., % two days	Subsequent treatment	Germination % after:				
			Days				
			2	4	6	8	10
Dark Lead foil and black paper	43.5	Intact { Red	0	3	20	47	93
		Clear	0	13	90*	90	90
		Clipped, dark	98		Thrown out		
Blue light	0.5	Intact { Red	0	10	50	86	98
		Clear	0	22	78	94	94
		Clipped, dark	98		Thrown out		

\* This test was a little moister than the others, although all appeared to have ample moisture for germination under normal conditions.

somewhat faster than red light. This contradicts a previous observation (Expt. 2) in which the reverse was the case, but it is probable that the metabolic drift already referred to may have changed the response of the seeds in this respect. Further, the intensity of the light was considerably higher in the 'clear' than in the red light tests and this would also contribute to the difference.

During the course of the above work, certain observations had brought up the question as to whether the time of day when the tests were set had any influence upon the results. The source of light had been daylight with its natural fluctuations. Accordingly Expt. 9 was carried out to test this. Eight lots of 100 seeds were planted on uniform blotters uniformly moistened in covered Petri dishes and wrapped in blue cellophane. Four lots were planted at 9 p.m. and four lots at 9. a.m. Table III presents the results:—

TABLE III  
EFFECT OF DAY PRECEDING NIGHT AND NIGHT DAY

Test	Planted	Period	Germination	Significance*
a	p.m.	3 N 2 D	1	$\chi^2 = 19.606$ $n = 1$
b	p.m.	3 N 3 D	1	
c	p.m.	4 N 3 D	2	
d	p.m.	4 N 4 D	1	
e	a.m.	3 D 2 N	12	$P < .01$
f	a.m.	3 D 3 N	4	
g	a.m.	4 D 3 N	9	
h	a.m.	4 D 4 N	6	

\* Fisher, R. A. *Statistical methods for research workers*, 3rd ed. p. 70.

From these results it appears that the total period before the counts were made has no bearing on the germination results but that significantly higher results were secured when day preceded night than in the reverse case. All sprouts were well grown so that the seeds either grew uninhibited, having started early, or were completely inhibited. The test of significance was based on the assumption that the N-D tests could be taken as a group having produced five sprouts out of 400 seeds and the D-N tests as another group with 31 sprouts out of 400 seeds. The value of  $P$  is so small that the two results cannot be considered as random deviates of the same population.

Working on the assumption that the blue light effects were due to an inhibitor soluble in water and capable of diffusion away if sufficient water was present, Expt. 11 was carried out. In this experiment 100 seeds were set in blue light using the same technique as in the preceding except that excess water was added so that the seeds were almost floating. In this experiment 15 sprouts were obtained in two days. During counting the seeds were exposed for about 30 sec. to diffuse daylight before being rewrapped in the blue cellophane. Two days later a total of 26 sprouts had been obtained and no further germination was secured. Since this test was planted at night and was thus an N-D test, for statistical purposes we may compare it with the N-D tests of Expt. 9. Here again  $P < .01$  and the difference is to be considered as significant.

Expt. 12 was designed to test further the influence of moisture. No exact control of moisture was attempted, but with the dark gray blotter used, which becomes very dark on being thoroughly wetted, it was an easy matter to arrange the blotters in order of increasing depth of shade corresponding to increasing amounts of moisture. Initially three degrees were used, slight moisture, medium, and wet. The first was secured by quickly plunging the blotter into water and withdrawing it immediately, medium was with the blotter fully soaked, and wet was with sufficient additional moisture added to make the surface appear quite wet, with free water at the edges. At the end of the experiment it was found that slight drying had occurred, and that somewhat unevenly, so that it was possible to differentiate five degrees of moisture. In each of the three initial degrees of moisture, 50 seeds were set in dark and 50 in red light. The results follow in Table IV. Expt. 11 is also included.

These results clearly indicate that excess moisture is needed to secure complete germination, but that degrees of moisture that are limiting in dark are not so in red light. Germination remains incomplete even in free water in blue light.

Several points of special significance emerge from these preliminary tests. It is evident that, with the rupture of the testa, the agent conditioning dormancy is rendered inactive, while a similar effect is brought about by the presence of excess moisture, and that most readily in red light, least readily in blue light, and to an intermediate degree in the dark.



TABLE IV  
INFLUENCE OF THE DEGREE OF MOISTURE

Degree of moisture	Germination, %, in two days in:—		
	Red light	Dark	Blue light
1		8 (S)	
2	74 (S)		
3		14 (M)	
4	98 (M)		
5	98 (W)	100 (W)	(W) 15 (26 in four days)

*Initial moisture:* (S) = slight; (M) = medium, and (W) = wet.

These observations are all compatible with the postulation of an inhibitor that may escape on rupture of the testa or by leaching when excess water is present, and that is present to the greatest degree in blue light, and to the least degree in red light.

The experiments in which seeds set to germinate in blue light were subjected either to light first or to darkness first demonstrate that there is a critical time before which blue light experience is not effective. That complete germination was not secured in those cases where light preceded darkness is to be ascribed to the probability that the critical period had been passed by the majority of the seeds before the initial light experience ended.

The results so far secured led to a tentative hypothesis that the effect of light was to render the semipermeable seed coat permeable, permitting the escape of an inhibitor that normally developed during the initial stages of germination, red light being specially active in this regard and blue light, not only inactive, but, if anything, tending to render the seed coat somewhat less permeable. Carbon dioxide suggested itself as a possible inhibitor; if it were, seeds set to germinate in an atmosphere of pure carbon dioxide would be expected to become dormant. In the next two sections these suggestions were explored.

## II. Further Experiments on Germination of Lettuce Seeds

### *Expt. N.S. VII*

In the respiratory flasks described in Section V, two grams of seed were set at 20° C., with humidified circulating air in blue light. The light failed at an unknown time but evidently had been adequate since no germination occurred up to 89 hr. After this treatment the seeds were dried at room temperature in the light and two lots of 100 seeds each were pricked and set to germinate at 20° C. in clear light; a further 200 seeds, unpricked, were set to germinate immediately.

### *Expt. N.S. VIIa*

A similar experiment was carried out, but in clear light, and the respiratory chamber was filled with carbon dioxide and sealed. After 94 hr. continuous

light, germination was found to have been completely inhibited. The seeds were then treated exactly as in N.S. VII.

The results of Expts. N.S. VII and N.S. VIIa are shown in the graphs, Fig. 2.

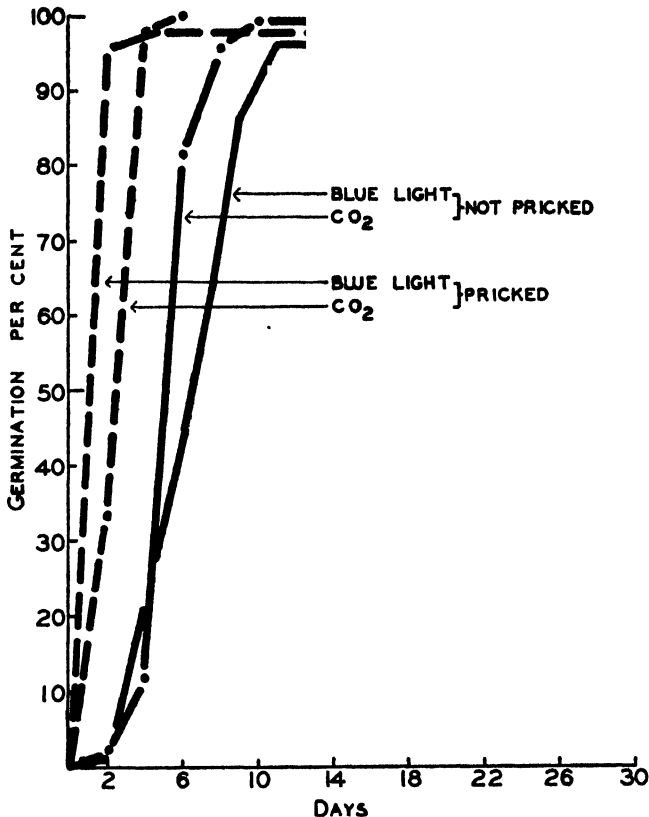


FIG. 2. Experiments N.S. VII and N.S. VIIa—germination in light of seeds pretreated with blue light and carbon dioxide.

#### Expt. N.S. XIV

This experiment was practically a repetition of Expt. N.S. VII, except that 1 gm. of seed was set in duplicate at 20° C., for 96 hr. in continuous blue light. From each lot, 2 × 75 seeds pricked and 2 × 75 unpricked were set to germinate in the dark at room temperature (close to 20° C.), and the same number pricked and unpricked were set to germinate in daylight. The corresponding replicates all gave very concordant results, the averages being shown in the graphs, Fig. 3. Results are shown up to 91 days. Ungerminated seeds still appeared fresh after this time.

### III. Experiments to Determine Changes in Fats

Since the layer responsible for the semipermeability of the testa of lettuce seed is lipid in character, any increase in permeability would be expected to be due to lypolysis, a process that could be detected by increase in free acid.

For this purpose a series of experiments was carried out to determine, by the methods commonly employed to determine saponification and acid numbers, and ester values, the amounts of free acid, total fatty acid and fats, all expressed as oleic acid radical per gram of seed, in dry seeds and in seeds after being exposed to germination conditions in blue light and in clear light. It was found, however, that the amount of fats in lettuce seeds is so high (36.6 to 39.9% according to my analyses) that changes in the seed coats evidently could not be traced by this method.

*Expt. N.S. XVIII*

In a further attempt to trace such changes, seeds were sectioned dry and also after imbibition in clear light and in blue light. The conspicuous semi-permeable membrane of the inner epidermis of the integument described by Borthwick and Robbins (1), gave the characteristic orange color of suberin

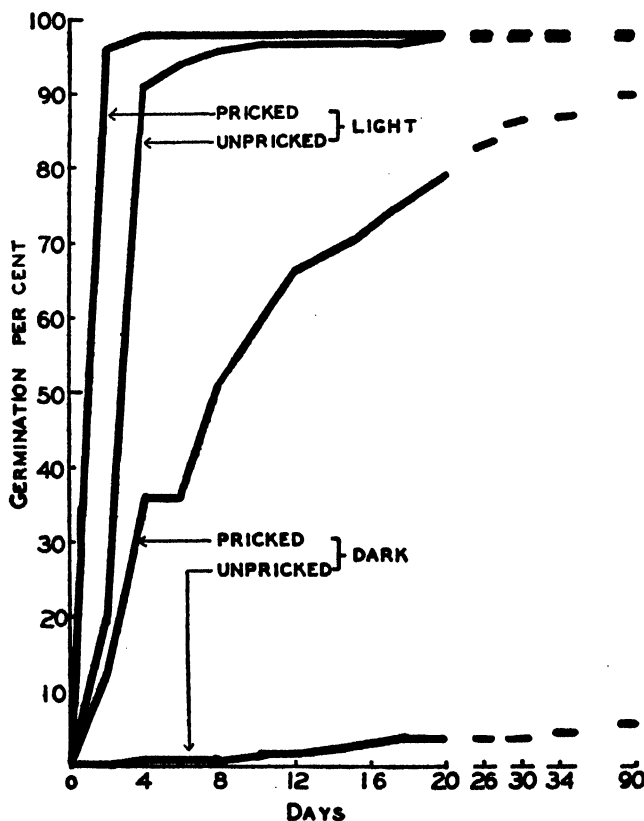


FIG. 3. *Experiment N.S. XIV—germination in light and darkness of seeds pretreated with blue light.*

with Sudan III. With Nile blue sulphate, after differentiation with 2% acetic acid, neither the characteristic pink of fats nor the blue of free fatty acids could be detected in any of the preparations.

#### IV. Discussion of Expts. N.S. VII, N.S. VIIa, and N.S. XIV

Results of Expts. N.S. VII, N.S. VIIa, and N.S. XIV have shown:—

(1a). That after seeds have become blue light dormant, rupturing the testa by clipping or pricking permits germination to proceed.

(1b). That after 24 hr. by blue light treatment followed by drying, however, unpricked seeds germinated almost as fast as pricked seeds in clear light, whereas in the dark, even pricked seeds showed marked retardation, while unpricked seeds in the dark germinated only to a very low percentage. It should be remarked here that the blue light pretreatment of seeds in N.S. XIV was much more intense than in Expt. 6, which may account for the slower germination of pricked seeds in the dark in the former than in the latter.

It seems a legitimate conclusion from this that the effect of blue light is not primarily upon the seed coat, since if it were, we should have expected little difference between germination in light and in darkness once the testa had been ruptured by pricking. The fact that adequate seed ventilation is provided by rupturing the testa is indicated by the prompt germination of clipped seeds in Duco dishes in which unclipped seeds were inhibited from germinating, as was shown in the preliminary section. This conclusion is further supported by the negative result of Expt. N.S. XVIII.

(2). Carbon dioxide, at a concentration of 100%, inhibits germination.

Germination curves for pricked and unpricked seeds in light resemble similar curves for blue light—treated seeds far more closely than the latter resemble those of blue light—treated seeds in the dark. (Compare graphs for Expts. N.S. VII and N.S. XIV, Figs. 2 and 3.)

Thus, while the effect of carbon dioxide is not identical, it is at least similar to that of blue light—probably a difference of degree rather than kind.

#### V. Respiratory Studies with *Lactuca sativa* L.

##### MATERIAL

Respiratory studies were carried out on a particularly good sample of Hansen lettuce of high germination (99 to 100%), with large plump seeds and of the 1934 crop, thus past that period in its metabolic drift that would make light a necessary requirement for satisfactory germination.

##### METHODS

##### *The Respiratory Chamber*

Exact details of the respiratory chambers need not be given. Erlenmeyer flasks (250 cc.) were used and so arranged that samples of the respired gases could be withdrawn without change of pressure and the whole chamber could be flushed out with fresh, humidified air when needed. The volumes of the chambers were determined to an accuracy of 0.5%, allowance being made for seeds, filter paper, and added water.

At the bottom of each flask was placed a circular filter paper, the proper amount of water added, and the seeds sprinkled in an even layer over it.

### *Measurement of Respiration*

The respired gases were analyzed by means of a Haldane respirometer using a gas burette graduated in 1 cc. units up to 7 cc. thereafter in 0.01 cc. units up to 10 cc. Readings could be made by estimating to 0.001 cc. Temperature fluctuations were automatically compensated by a dummy burette, the two burettes being enclosed in a water jacket. Barometric fluctuations were negligible during the course of a determination.

### PROCEDURE

In all cases exactly 2 gm. of seed was used. The seeds having been spread as described on the wetted and drained filter, a measured volume of water was added (6 cc. in the first experiments, 3 cc. subsequently), and the flasks were then flushed for five minutes. The air used for flushing, taken either from outside or from the compressed-air lines, was analyzed at frequent intervals, these analyses being used as blanks.

The results of analysis were calculated to cubic centimeters per gram per hour. Duplicate analyses were made in all cases and where reasonably close agreement was not secured, additional analyses were made. In addition, each entire experiment was done in duplicate although, as will be seen, it was not possible always to duplicate an experiment exactly.

The respiratory chambers were wrapped in red, blue, or clear cellophane before being placed in constant temperature tanks for the period of respiration. The light source was a 250 w. Mazda lamp placed in a glass cylinder immersed in the tank, and spaced about 6 in. from the respiratory chambers.

Tests with red and clear light could only be carried out during a limited time, since the seeds germinated and thus the respiration ceased to be characteristic of seeds. Blue light tests, however, were prolonged since germination was completely inhibited.

The work was done during September, October, and November, 1935.

### RESULTS

#### *Expt. R I*

Seeds allowed to imbibe 14 hr. with constant illumination in sealed chamber and respired air analyzed. The following results were secured, expressed as percentages and in cubic centimeters per gram per hour. The temperature in the constant temperature tank was 20° C.

Color of light	Replicate analysis	CO <sub>2</sub>		O <sub>2</sub>		R. Q.
		%	Cc. per gm./hr.	%	Cc. per gm./hr.	
Blue	1	2.84	0.2121	3.60	0.2697	0.787
	2	2.87		3.66		
Clear	1	3.67	0.2788	4.46	0.3412	0.817
	2	3.66		4.51		

*Expt. R Ia*

This was a duplicate of the above except that the period of respiration was 16½ hr. The temperature control failed during the respiratory period and at the end the temperature was 23° C., having started at 20° C. The results follow:

Color of light	Replicate analysis	CO <sub>2</sub>		O <sub>2</sub>		R. Q.
		%	Cc. per gm./hr.	%	Cc. per gm./hr.	
Blue	1	3.64	0.2353	4.36	0.2501	0.839
	2	3.65		4.33		
Clear	1	5.53	0.3485	6.37	0.4024	0.866
	2	5.53		6.40		

*Expt. R II*

This was a further replicate of Expt. R I as far as the blue light was concerned, but instead of clear, red cellophane was used. The period of respiration was 14 hr. and the temperature, while the control was still somewhat uncertain, was between 20° C. and 21° C.

Color of light	Replicate analysis	CO <sub>2</sub>		O <sub>2</sub>		R. Q.
		%	Cc. per gm./hr.	%	Cc. per gm./hr.	
Blue	1	2.95	0.2244	3.71	0.2840	0.790
	2	2.95		3.76		
Red	1	3.93	0.2914	4.83	0.3592	0.810
	2	3.92		4.84		

*Expt. R III and Expt. R IIIa*

In these, blue and red were again the colors used but the respiratory period was reduced to four hours. Owing to several mishaps only one analysis has been accepted from Expt. R III since it checks with the analysis in Expt. IIIa. Temperature control was satisfactory at 20.25° C. The one test in R III was with blue light. The following are the results of these two experiments:—

Color of light	Experiment and replicate	CO <sub>2</sub>		O <sub>2</sub>		R. Q.
		%	Cc. per gm./hr.	%	Cc. per gm./hr.	
Blue	R III 1	0.86	0.2437	0.98	0.2727	0.895
	R IIIa 1	0.91		1.00		
Red	R IIIa 1	0.97	0.2636	1.04	0.2851	0.920
	2	0.99		1.08		

*Expt. R IV*

This was similar to the previous experiments with a respiratory period of four hours but with red and clear cellophane. Temperature control was satisfactory at 20° C. to 20.25° C. The results follow:—

Color of light	Replicate analysis	CO <sub>2</sub>		O <sub>2</sub>		R. Q.
		%	Cc. per gm./hr.	%	Cc. per gm./hr.	
Red	1	1.01	0.2688	1.19	0.3168	0.85
	2	1.01		1.18		
Clear	1	1.10	0.2964	1.24	0.3224	0.89
	2	1.11		1.24		

*Expt. R V*

This experiment constituted in part a duplicate of RI and in addition the blue light test was continued for 95½ hr., being sampled at intervals. In this experiment the respiratory flasks were flushed with air after each sampling and remained sealed until the next sample was drawn. Thus the respiratory periods sampled vary considerably. The temperature was 20.75° C. at the start and 20.5° C. at the end. Results:

Color of light	Analysis	Respiratory period, hr.	Total time elapsed	CO <sub>2</sub>		O <sub>2</sub>		R. Q.
				%	Cc. per gm./hr.	%	Cc. per gm./hr.	
Clear		3.5	3.5	0.94	0.2833	0.985	0.2969	0.95
Blue	a	3.5	3.5	0.91	0.2808	0.98	0.3024	0.93
"	b	3.5	7.1	1.135	0.3502	1.32	0.4073	0.86
"	c	16.2	23.3	3.335	0.2227	4.16	0.2778	0.80
"	d	3.5	27.0	0.85	0.2623	1.03	0.3024	0.83
"	e	44.5	71.5	6.32	0.1535	7.91	0.1906	0.80
"	f	3.5	75.1	1.16	0.3579	1.16	0.3579	1.00
"	g	3.5	78.7	0.93	0.2870	1.09	0.3363	0.85
"	h	16.7	95.5	3.71	0.2401	4.59	0.2974	0.81

*Expt. R VI*

Owing to the strongly depressive effects observed due to increasing accumulation of carbon dioxide and diminution of oxygen in the long respiratory periods in the preceding experiment, another experiment was carried out in blue light similar to R V except that the flasks were continually flushed with humidified air until sealed for the respiratory period. The temperature varied between 20° C. and 20.25° C. during the experiment. Results:—

Analysis	Respiratory period, hr.	Total time elapsed	CO <sub>2</sub>		O <sub>2</sub>		R. Q.
			%	Cc. per gm./hr.	%	Cc. per gm./hr.	
<i>a</i>	2.8	19	0.665	0.2476	0.80	0.2979	0.83
<i>b</i>	3	22.2	0.585	0.2057	0.735	0.2602	0.79
<i>c</i>	3	43	0.38	0.1336	0.71	0.2497	0.54
<i>d</i>	3	46.2	0.44	0.1547	0.73	0.2567	0.60
<i>e</i>	3	49.4	0.44	0.1547	0.69	0.2427	0.64
<i>f</i>	3	52.6	0.49	0.1723	0.635	0.2233	0.77
<i>g</i>	3	67.1	0.395	0.1389	0.67	0.2356	0.59
<i>h</i>	2.75	114.8	0.57	0.2187	0.96	0.3683	0.59

*Note: During entire respiratory period, seeds held in blue light.*

#### *Expt. R VIa*

This was a repetition of the previous experiment. Results are tabulated below:—

Analysis	Respiratory period, hr.	Total time elapsed	CO <sub>2</sub>		O <sub>2</sub>		R. Q.
			%	Cc. per gm./hr.	%	Cc. per gm./hr.	
<i>a</i>	3	19	0.47	0.1653	0.75	0.2637	0.63
<i>b</i>	3	22.1	0.65	0.2286	1.04	0.3657	0.63
<i>c</i>	3	43	0.415	0.1459	0.67	0.2356	0.62
<i>d</i>	3	46.1	0.57	0.2004	0.85	0.2989	0.67
<i>e</i>	3.6	49.9	0.73	0.2120	1.12	0.3252	0.66
<i>f</i>	2.8	52.8	0.73	0.2767	1.07	0.4056	0.69
<i>g</i>	2.75	66.8	0.325	0.1247	0.49	0.1880	0.66
<i>h</i>	3	90.8	0.665	0.2339	1.09	0.3922	0.61

*Note: During entire respiratory period, seeds held in blue light.*

In Table V, Expts. R V to R VIa are summarized in such a way as to show chronological relationship, the figures in bold face type being those that are used for plotting the graph, Fig. 4, and that have been selected for the following reasons. With regard to R V, it has already been mentioned that prolonged periods with the seeds in sealed vessels resulted in a marked depression of the respiratory rate. For this reason, only those results are accepted that represent data for short respiratory periods. Examination of the data for Expts. R VI and R VIa shows that in general lower values for oxygen, carbon dioxide, and respiratory quotients are obtained after long periods of ventilation than after short periods of ventilation such as those between respiratory periods succeeding one another at short intervals. The constant occurrence of this phenomenon suggests that a cumulative factor other than blue light is operating to give progressively higher values until a long ventilating period intervenes to alleviate the condition. For this reason only those values are accepted that represent data obtained after a long period of ventilation. Results secured after 70 hr. are not accepted since mold growth became evident after this time.



TABLE V  
SUMMARY OF EXPERIMENTS R V, R VI, AND R VIa

	Hours											
	3.5	7.1	19	22.1	23.3	27	43	46.1	49.6	52.7	67.0	71.5
R V	.281	.350			.223	.262						.154
CO <sub>2</sub> R VI			.248	.206			.134	.155	.155	.172	.139	
R VIa			.165	.229			.146	.200	.212	.277	.125	
R V	.302	.407			.288	.302						.191
O <sub>2</sub> R VI			.298	.260			.250	.257	.243	.223	.236	
R VIa			.264	.366			.236	.299	.325	.406	.188	
R V	.93	.86			.80	.83						.80
R.Q. R VI			.83	.79			.54	.60	.64	.77	.59	
R VIa			.63	.63			.62	.67	.66	.69	.66	

Examination of the graph, Fig. 4, reveals substantially a drift with time from higher initial values to lower final values both for carbon dioxide output, for oxygen uptake, and for respiratory quotient. The fact that the curves are, in general, at somewhat different levels may be ascribed to two causes; variations introduced by experimental and sampling errors and the fact that the experiments were separated by a considerable interval of time, Expt. R VIa having been carried out a month later than the other two; thus the samples may not have been at comparable stages in their metabolic drift. Bearing this in mind, the fact emerges that the final values reached at 70 hr. are of the same order of magnitude, and the shape of the curves suggests that the measures of respiration rate and of respiratory quotient are approaching values characteristic of blue light dormancy.

The curves given by R V may be regarded as representing initial stages of curves similar to those given by R VI and R VIa, and the differences between the curves as differences in the mode of progress to the same state exhibited by the several samples. These ideas may be schematized as in Fig. 5.

The work of Leach and Dent (4) on the respiration of fatty seeds has shown that the respiration rate of uninhibited seeds starts in the manner indicated by the upward slope of the curves shown in the above scheme, but that instead of the depression that begins at the seven hour point, the respiration continues to rise for a long time. This would indicate that the seven hour point represents a critical stage in the metabolic drift of lettuce seeds at which blue light begins to be operative, and is in striking agreement with the failure of blue light to produce complete inhibition when blue light experience is stopped before this stage is reached, as shown by the preliminary experiments reported in Section I. It should be mentioned that the seven hour point

is not an exactly determined one and that the true critical point may lie somewhat on one or the other side of it, or more probably over a more or less extended period owing to statistical differences in the responsiveness of different seeds to germinating conditions.

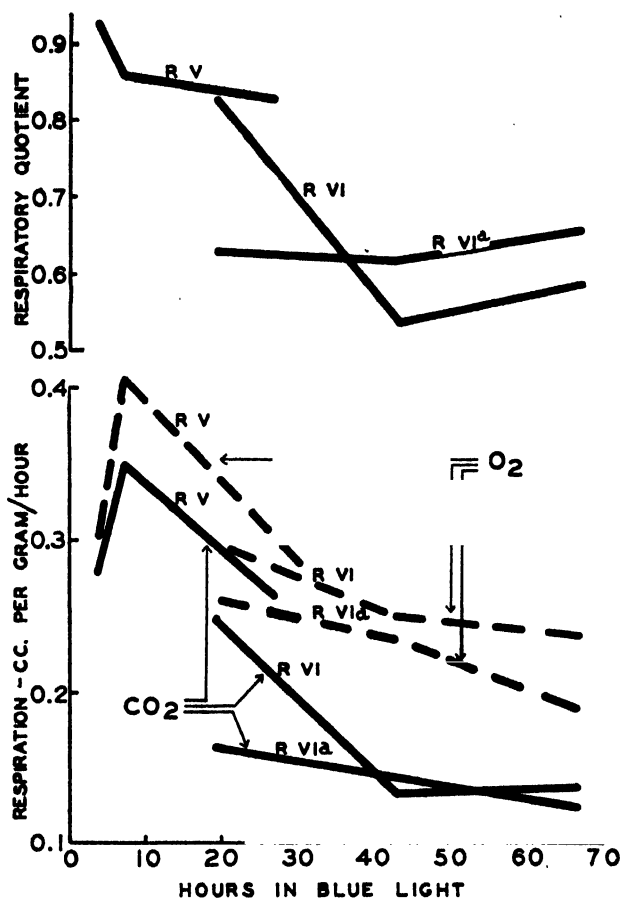


FIG. 4. Respiration of lettuce seed in blue light.

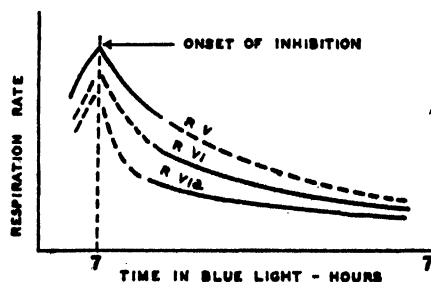


FIG. 5. Modes of progress of different samples to respiration rates characteristic of blue light dormancy. Schematic.

It is of interest to compare the drift of respiratory quotient in the case of inhibited lettuce seeds with that of uninhibited seeds as given by Leach and Dent. For this purpose we may use the results they give for seed of *Helianthus annuus*, belonging to the same botanical family as lettuce. In the graph, Fig. 6, are shown our own results for R V and R VI, which were done within a few days of each other, superposed upon theirs and reduced to the same percentage time scale. In both cases there is seen a fall to a minimum fol-

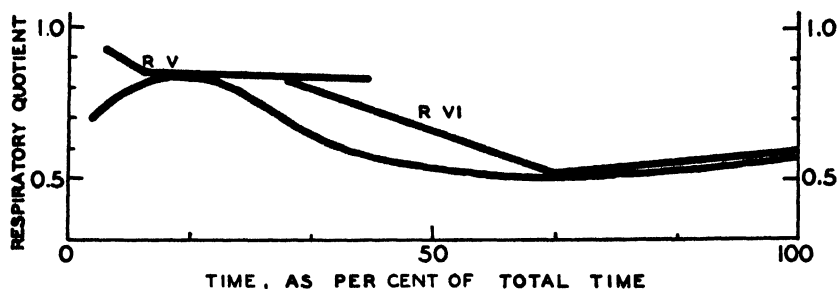


FIG. 6. Drift of respiratory quotient for lettuce seeds compared with that for sunflower. Curve for sunflower from Leach and Dent.

lowed by a gentle rise and also the absolute magnitudes are closely similar. The same gentle rise is also given by R VIa, but at a slightly higher level. For the time being, and in the absence of similar determinations on uninhibited lettuce seed, it is reasonable to suggest that the drifts of respiratory quotient in dormant and non-dormant lettuce seed are similar, while the curves of respiration deviate from one another sharply. This would point to an effectively equivalent depression of the pre- and postglycolytic phases as characteristic of blue light dormancy. It is evident that to substantiate this suggestion, more respiratory data are needed.\*

The results of the experiments recorded up to the present point led to the exploration of an hypothesis based upon the effect of light upon the respiratory mechanism. This hypothesis postulated an anaerobic zymasis induced by blue light, but further consideration seems to indicate that anaerobic zymasis is improbable under the conditions existing within the seed, where there is no reason to suppose oxygen tensions below the extinction point exist. However, the hypothesis led to the experiments next to be recorded, and further discussion will be delayed until after these have been considered.

\* The question whether oxidative anabolism may play a part in the respiration of lettuce seeds and if so, whether the effect of blue light might change the fate of the products of OA (oxidative anabolism) was considered. It seems reasonable to suppose that the carbon that would be lost in the absence of OA, when anabolized may be incorporated as structural carbon. If the critical seven hour period should coincide with the initiation of growth processes with consequent demand for structural carbon, and if the products of OA are diverted from this goal by blue light, we might here find the explanation of the inhibition produced by blue light. In the absence of evidence that OA exists in the material in question we cannot develop this idea further at the present time, but suggest that it is a possibility worth following up.

## VI. Experiments to Induce Dormancy by Means Other than Blue Light

The hypothesis just mentioned suggested that the inhibiting substance was one of the products of anaerobic respiration.

This possibility was tested in the experiments that follow:

### *Expt. N.S. XIX*

Two lots of 100 seeds were set in light and  $2 \times 100$  in the dark, on top of filter papers in covered Petri dishes at  $20^{\circ}\text{C}$ . The filter papers were severally moistened with solutions of ethyl alcohol (1%), acetic acid (1%), and acetaldehyde (0.2%), and also glycerine 1% and 3%, using  $2 \times 100$  seeds in each case, but in light only. A check test was set between blotters in the dark at  $20^{\circ}\text{C}$ .,  $2 \times 100$  seeds being used. Thus, in all,  $18 \times 100$  seeds were used in this experiment. In moistening the filter papers, they were completely dipped in the solution until fully soaked and, after allowing the free water to drain off, were transferred to the Petri dishes. The seeds were then planted and 10 drops of solution added. The covered Petri dishes were then placed in humid germinators, the light tests being exposed to daylight, the dark tests being wrapped in sheets of wet, dark blotting paper and placed in dark chambers.

### *Results*

*Check tests*.—Germination was practically complete (94.5%) in 46.5 hr.; 44.5 hr. later 2% more had germinated, and 23 hr. later another 0.5% germinated. The remaining 3% apparently went into dormancy, since no further germination was secured after 50 days, although the seeds still looked healthy.

*Acetaldehyde* (0.2%).—Germination was apparently somewhat stimulated at this concentration, since all good seeds germinated in 48 hr., a total of 98.5% in light and 99.5% in dark. Within the next two days, one weak sprout had developed in the light test and 23 days later another weak sprout had grown, while the remaining seed was evidently dead. The single ungerminated seed in the dark test was dead.

*Acetic acid* (1%).—The seeds were all killed and had swelled to twice their normal size.

*Ethyl alcohol* (1%).—This solution showed marked retardation of germination, both in light and dark. The latter gave somewhat conflicting results in the duplicates, so two more replicates of 100 seeds were run. These concurred well with each other and also with one of the other replicates, thus the three concordant tests were accepted.

Detailed results are shown on Fig. 7.

*Glycerine* (1% and 3%).—Germination was depressed by the 1% solution and almost completely inhibited by the 3% solution (2.5% germination) but the dried seeds showed no signs of dormancy.

*Expt. N.S. XIXa*

In this experiment  $4 \times 100$  seeds were set (the same technique being followed), two in light and two in dark, using a solution of 2% ethyl alcohol. At this strength germination was almost completely inhibited as follows:—

Test	Seeds used	Sprouts after 49.5 hr.
Light 1	100	3
Light 2	100	0
Dark 1	100	1
Dark 2	100	0

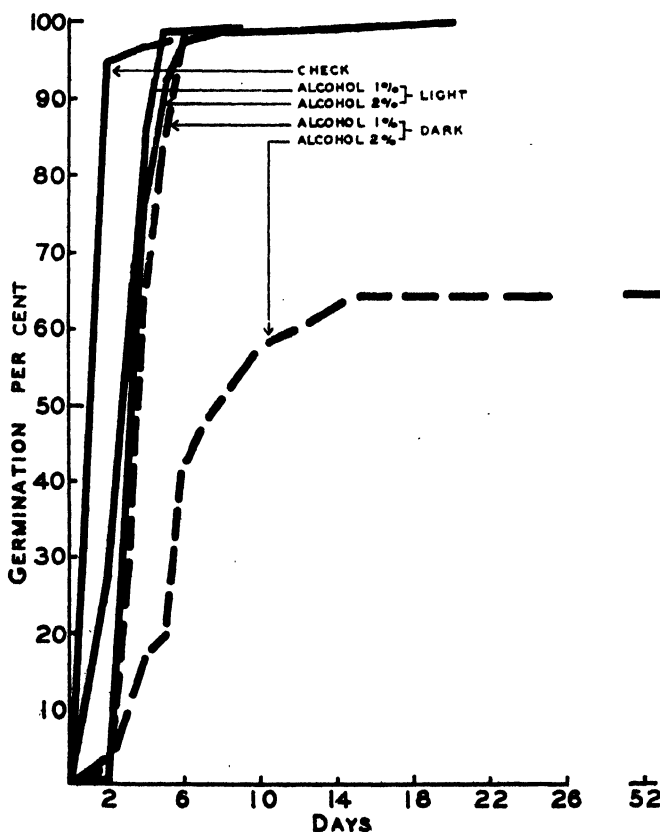


FIG. 7. *Experiment N.S. XIX—germination in light and darkness of seeds made dormant by alcohol.*

The ungerminated seeds were allowed to dry in the dark and were then replanted according to the following plan:

Original treatment	Subsequent treatment
(1) Light 1 } (2) Dark 1 }	{ Dark, between blotters moistened in tap water, in dark cabinet, at 20° C.
(3) Light 2 } (4) Dark 2 }	{ On top of filter papers under bell jars in Jacobsen apparatus, at 20° C., daylight.

Since the subsequent treatment evidently had much greater effect than the original treatment, and since (1) and (2) concorded reasonably well, and (3) and (4) very well, (1) and (2) have been averaged, as have (3) and (4) in the graph that is shown in Fig. 7.

Another experiment was also run using a concentration of 0.2% acetic acid. At this concentration the solution gave results almost identical with acetaldehyde, 0.2%; 98.25% germination was secured after 47 hr.

#### *Expt. N.S. XIXb*

This experiment was based upon the same general plan as the alcohol 2% test, but in this case 3% was used. At this strength inhibition of germination was complete. After the seeds had dried in the dark, 50 seeds of each replicate were pricked. Pricked and unpricked seeds were then replanted according to the following plan.

Original treatment	Subsequent treatment
(1) Light 1	P (pricked) Dark as in N.S. XIXa
(2) " 1	U (unpricked) " " "
(3) " 2	P (pricked) Light " " "
(4) " 2	U (unpricked) " " "
(5) Dark 1	P Dark " " "
(6) " 1	U " " " "
(7) " 2	P Light " " "
(8) " 2	U " " " "

Again, initial treatment seemed to have had little effect and excellent checks were given by (1) and (5), by (2) and (6), by (3) and (7), and by (4) and (8). These pairs have accordingly been averaged and the results are shown in Fig. 8.

#### *Summary of Experiments N.S. XIX, XIXa, and XIXb and N.S. XIV*

Expt. N.S. XIV showed that seeds treated with blue light under conditions otherwise suitable for germination, dried and then pricked, germinated as promptly in light as did the check test (Figs. 2 and 3). Similar seeds germinated in the dark showed marked retardation, but germination had reached the 90% point after 90 days. Unpricked seeds had germinated fairly promptly and completely in the light, but in the dark showed extreme retardation.

Expts. N.S. XIX, XIXa, and XIXb show that alcohol not only inhibits germination, but that the treated seeds have become dormant, even after drying. It is significant that this dormancy is coupled with light sensitive-ness of a type similar to, though less marked than, that induced by blue light.

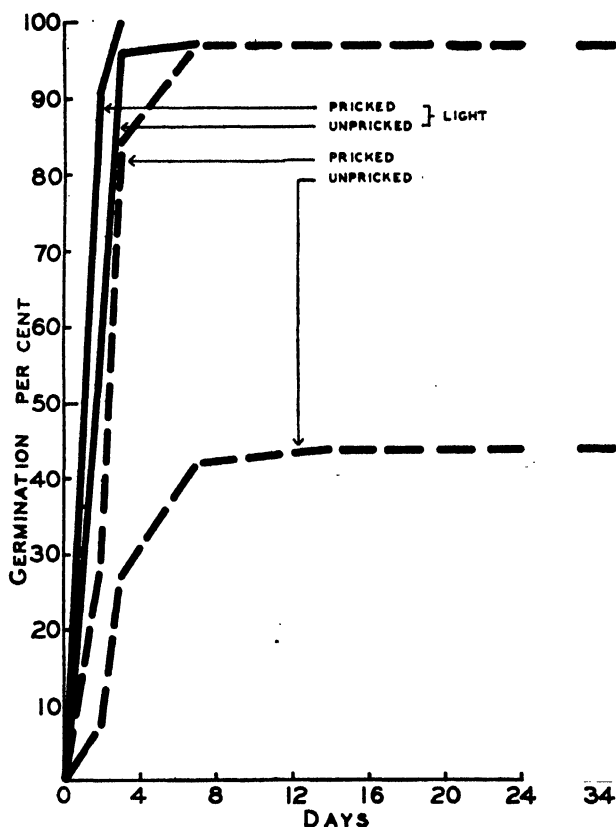


FIG. 8. *Experiment N.S. XIX (continued)*—germination in light and darkness of seeds made dormant by alcohol, 3%; pricked and not pricked.

#### *Expt. N.S. XX*

It is a well known fact that high temperature brings about dormancy in lettuce seeds. It was thought of interest to examine whether pricking would offset high temperature dormancy. Accordingly, the following experiment was carried out.

Two lots of 100 seeds, pricked, and 2 × 100 seeds, unpricked, were set between blotters in the dark at a constant temperature of 31° C.

After 46 hr., the unpricked seeds had all become dormant. Of the pricked seeds, two were evidently dead and were removed, while one seed had germinated. The tests were then transferred to low temperature (20° C.) still in the dark where they stayed for the remainder of the test. The results are shown in Fig. 9. The duplicates checked well with each other and have been

averaged. The pricked tests are shown as originating from zero co-ordinates, and have been calculated to percentage of ungerminated seeds at time of transfer to lower temperature.

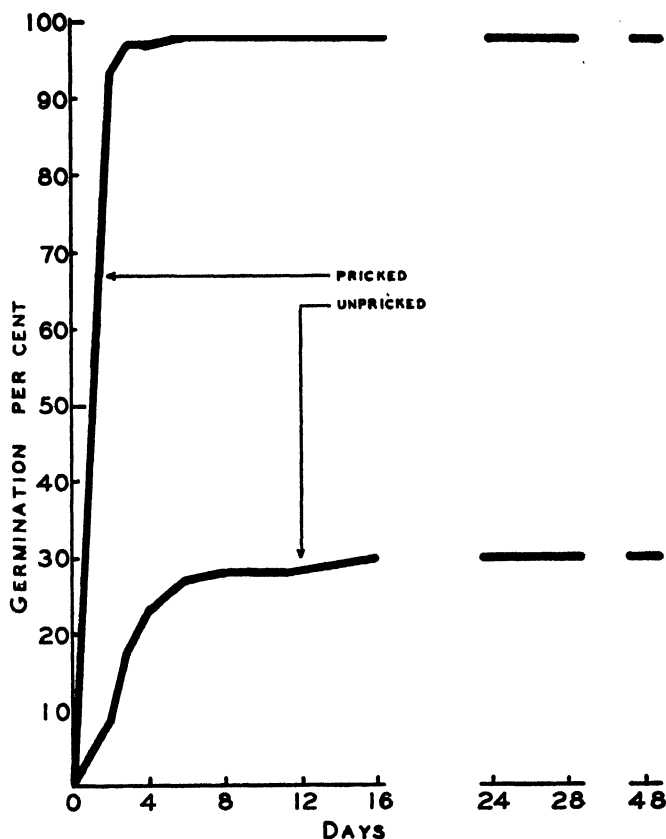


FIG. 9. Experiment N.S. XX.—germination in darkness of seeds made dormant by high temperature; pricked and not pricked.

## VII. Discussion of Metabolic Possibilities

Since we have felt compelled to reject the suggestion that an anaerobic type of zymasis is brought about by blue light, we may consider another possibility. We have seen that carbon dioxide and alcohol induce dormancy of a type similar to that induced by blue light. It is known that in the presence of carbon dioxide a type of zymasis proceeds that results (in the cases studied) in the production of much acetaldehyde and little alcohol and it is at least possible that a similar type of zymasis would be brought about by means of ethyl alcohol. These suggestions seem to indicate the possibility that the effect of blue light is to bring about a state of a similar type, either through the agency of carbon dioxide or in some other way.

Both on *a priori* considerations and on the grounds that a rising phase of respiratory quotient in the initial stages of germination is a perfectly general



phenomenon with fatty seeds, we may regard the metabolic state of the germinating seed as including a certain amount of zymasis such that under normal conditions some alcohol is produced. If the effect of blue light, as suggested above, is to bring about the same type of zymasis as does carbon dioxide, then we would expect a lower proportion of alcohol in seeds set to germinate in blue light than in those in clear or red light.

Expt. XXI, which was originally planned for another purpose, gives strong support to this hypothesis and is reported below.

#### *Expt. XXIb*

Lettuce seed (100 gm.) was spread in a thin layer on wet blotters lining the bottom of a shallow tank (37 in.  $\times$  19 in.  $\times$  3 in.), which was then covered by a sheet of glass on which was placed a double layer of dark blue cellophane. Above the tank at a height of  $4\frac{1}{2}$  ft. was suspended a 500 w. light. The seeds were continuously illuminated for 28 hr., since this experiment was to be checked by XXIc, in which seeds were similarly allowed to imbibe but in clear light, and in which it was desirable that the germination process should not be carried too far. The temperature was  $22.5^{\circ}$  C. above the cellophane. The alcohol content was determined by the Nicloux method. The seeds were distilled in picric acid and two aliquots of the distillate of 5 cc. each were used and titrated with a 19 gm. per liter solution of potassium dichromate after being mixed with an equal volume of concentrated sulphuric acid. Each aliquot required 0.35 cc. of the solution, which corresponded with an alcohol content of 0.008%.

In Expt. XXIc, carried out in precisely the same way as XXIb except for light color, which in the present case was unscreened, and the fact that the temperature was  $24^{\circ}$  C., each aliquot required almost exactly 1 cc. potassium dichromate, corresponding to 0.020% alcohol content.

The possibility that the lower alcohol content of the seeds used in XXIb is due to the lower temperature of that test must be mentioned. However, the difference is far greater than could be accounted for by an application of the Van't Hoff relation, which gives a corrected value for XXIb of 0.009 at  $24^{\circ}$  C., and we must conclude that a real difference exists. These results support the premise that some zymasis occurs in the normal, germinating seed, as also the suggestion that in blue light the character of the zymasis is changed in such a way that carbon dioxide zymasis predominates.

#### **Acknowledgments**

I have to make grateful acknowledgment to Dr. H. B. Sifton under whose guidance this study was carried out, and for whose encouragement and helpful criticism I am much in debt; to Dr. G. H. Duff, for his valuable criticisms and suggestions especially with regard to interpretation of results; to Prof. J. Rogers for help in the alcohol determinations; and also to members of the staff of the Department of Botany for discussions and suggestions that have been of much use.

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## A STUDY OF THE MYCELIUM AND HAUSTORIA OF THE RUSTS OF *ABIES*<sup>1</sup>

BY LILLIAN M. HUNTER<sup>2</sup>

### Abstract

A study of the mycelium and haustoria of certain rusts of *Abies* has been made. It was found that the mycelium of three rusts have distinguishing characteristics. The haustoria of *Melampsorella Cerastii*, *Hyalopsora Polypodii* and *Milesia polypodophila* are so distinctly characteristic as to serve as diagnostic criteria for the species. The haustoria of six *Uredinopsis* species are similar in type; those of two species of *Pucciniastrum* are of the same type. The haustoria of *Calyptospora Goeppertiana* are hyphal and very slender. The relation of the haustoria of the rusts, up to near maturity, is one of high symbiotic nature in *Abies balsamea*. The haustoria of *Milesia polypodophila* and certain other rusts in their aecial hosts show a contrast in type to the haustoria in their telial hosts. The haustoria of *Milesia Scolopendrii* and *M. vogesiaca* are hyphal or of loose spiral form. The haustoria of *M. Kriegeriana* resemble those of *M. intermedia*. The haustoria of *M. Polypodii* are usually characteristically branched, serving as an aid in identification. Haustoria were found to occur in the leaves of 19 species of the Pucciniastreae on *Abies*. Eighteen of these are described in the aecial host for the first time.

### Introduction

Interest in the occurrence and nature of haustoria in the Uredinales has been growing gradually since DeBary (1) discovered the haustoria of *Melampsorella Caryophyllacearum* (*Aecidium elatinum*) on its systemic mycelium in the stem of *Abies pectinata*. Schroeter (21) made a search for haustoria in the same species on its telial host in 1874, but did not succeed in finding any. He described hyphae with short disclike swellings on the ends and stated definitely that the hyphae twist around the parenchyma cells tightly, pressing but not going inside them.

Sappin-Trouffy (19), in 1892, examined the mycelium and found haustoria in a single species for each of the genera *Puccinia*, *Uromyces*, and *Coleosporium*. He (19) expressed his intention of later giving a description of the haustoria for all the species he had met with and stated: "ils nous suffisait ici d'établir l'existence générale de ces organes dans la famille des Uredinées". In 1896 Sappin-Trouffy (20) described haustoria for nine species in a number of genera of the Uredinales.

Magnus (11), as early as 1899, described complicated haustoria for *Melampsorella Caryophyllacearum* Schroet. in the telial host. In 1901 he (12) stated

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that he had never been able to observe haustoria for *Milesia Kriegeriana* (Magn.) Arth. (*Melampsorella Kriegeriana* Magnus) (12) on the telial host, and in 1902 he (13) made a similar statement in connection with *Milesia Feurichii* (Magnus) Faull (*Melampsorella Feurichii* Magnus) (13). Magnus, in 1892, described the haustoria of *Hyalopsora Polypodii* (Pers.) P. Magn. In the same year he (9) reported that he had observed no haustoria on the mycelium of *Uredinopsis filicina* (Niessl.) P. Magn. Again in 1904 he (14) stated that the mycelium of the *Uredinopsis* species that he had studied is strictly intercellular and bears no haustoria. Faull (6) in an address presented before the International Congress of Plant Sciences, Section of Mycology, Ithaca, N.Y., Aug. 20, 1926, reported the finding of haustoria in the genus *Uredinopsis* for the aecial hosts by the writer, and for the telial hosts by Moss. Moss (16) described haustoria for *U. Atkinsonii*, *U. Osmundae*, and *U. Pheopteridis* in the telial hosts. Faull (6) also reported the finding, by Moss and the writer, of the haustoria of a number of other species in many of which they were noted for the first time. The writer's investigations of the haustoria have been continued and extended to a study of 19 species of the Pucciniastreae.

### Material and Methods

The material from which the sections of *Uredinopsis macrosperma* were made was obtained from the herbarium of the Department of Botany, University of Toronto, and was originally collected in the Western States. The European material of *Melampsora Abieti-Capraearum* and *Hyalopsora Polypodii* was obtained from Switzerland through Dr. Eugène Mayor. The rest of the material, with one exception, *Melampsorella Caryophyllacearum*, was obtained from culture experiments. These were supplemented by field collections.

The work of collecting and culturing most of the material was directed by Prof. J. H. Faull, who was assisted by his students, Dr. G. D. Darker, Dr. W. R. Watson, Dr. E. H. Moss, Dr. E. Bensley, and the writer; *Milesia Scolopendrii*, *M. Polypodii*, *M. vogesiaca*, and *M. Kriegeriana* were cultured by the writer in England. Culture experiments also made by the writer were carried on at the University of Toronto with the purpose in view of obtaining young spermogonial material of *Melampsora Abieti-Capraearum*; those carried on by the other students, above mentioned, were made in the Timagami Forest Reserve. All the material examined was of infected leaves of *Abies*, except in the case of *Milesia polypodophila*, in which the stem, as well as the leaves, was examined for mycelium and haustoria.

The fixatives used were the same as those named by the writer (8) in connection with a study of the spermogonia of the rusts of *Abies*, except that form-acetic-alcohol was added to the list.

The preparation of the material, with one exception, was also similar, and the same stains were used as those listed by the writer in (8). The exception referred to was in the treatment of the stem of *Abies* in which the mycelium of *Milesia polypodophila* is systemic. This species presented a problem in that there was difficulty in showing clearly the entrance of the haustorium into the cells of the cortex in the stem. Infected stems with leaves attached were fixed in medium chrom-acetic solution, treated in the usual manner, and embedded in celloidin. Sections of  $7\mu$  to  $15\mu$  in thickness were made by the use of the sliding microtome. The sections were taken through the usual grades of alcohols, the celloidin was dissolved, and the sections were then led, in graded solutions from 95% alcohol or 70% alcohol, to a lactophenol solution. Stains of acid fuchsin, cotton blue, and light green, each dissolved separately in lactophenol, were prepared. The amount of stain used in each case was just sufficient to give a deep color to the lactophenol. The clear solution of lactophenol applied to the section was drained off, and each of the three stains was applied separately to the sections of the diseased woody stem and leaf. The application was made by means of a pipette. The sections, covered by a bell jar for protection, were allowed to stand overnight. On the following morning the stains were removed by washing with a clear lactophenol solution. Very little color was left in the last solution and the objects were mounted with cover glasses. Preliminary examinations showed the sections thus treated to be promising for study, so in a few days after some evaporation had taken place the slides were 'ringed' with amber cement and put aside. When the cement had hardened the sections were examined under an oil immersion lens. The trial method proved satisfactory. The haustoria stood out more clearly than with the iron-alum haematoxylin or any of the other methods of staining used. The lactophenol mount was more satisfactory than any previously made in Canada balsam. The only parts of the section that took the stain were the haustoria, the intercellular mycelium and the contents, and the host cell nuclei. The general stain, which affected the entire section when iron-alum haematoxylin was used, was eliminated. There was an added value too, in that the lactophenol made the walls of the cortical cells quite transparent so that even in thick sections the narrow portion of the mycelium penetrating the host cell and connecting the intercellular mycelium with the haustorium proper was easily viewed. Cotton blue proved a good general stain for the mycelium. Acid fuchsin showed up the nuclei of the mycelium well. Light green, on account of its brightness, transparency, and its equal staining capacity, was preferable to the other stains used. Starch grains, cytoplasm, and the walls of the mycelium showed up brightly and very slightly colored in the preparations. The great value of the method was in demonstrating so clearly the penetration of the wall of the host cell by the mycelium.

## Description

The mycelium and haustoria described belong to the following species:

1. *Melampsora Abieti-Capraearum* Tubeuf.\*
2. *Melampsorella Caryophyllacearum* Schroet.
3. *Pucciniastrum Epilobii* (Pers.) Otth.\*
4. *Pucciniastrum Abieti-Chamaenerii* Kleb.\*
5. *Calypsotheca Goeppertiana* J. Kühn.\*
6. *Hyalopsora Aspidiotus* (Pk.) P. Magn.\*
7. *Milesia intermedia* Faull\*
8. *Milesia marginalis* Faull & Watson\*
9. *Milesia polypodophila* (H. P. Bell) Faull\*
10. *Milesia Scolopendrii* (Fuckel) Arth.\*
11. *Milesia Polypodii* White\*
12. *Milesia vogesiaca* (Syd.) Faull\*
13. *Milesia Kriegeriana* (Magn.) Arth.\*
14. *Uredinopsis Atkinsonii* P. Magn.\*
15. *Uredinopsis Phegopteridis* Arth.\*
16. *Uredinopsis Osmundae* Magn.\*
17. *Uredinopsis mirabilis* (Peck) Magnus\*
18. *Uredinopsis Struthiopteridis* Störmer\*
19. *Uredinopsis macrosperma* (Cooke) Magn.\*

All of the species listed above belong to the subfamily, the Pucciniastreae of the family Melampsoraceae. The first nine species named above and those from 14 to 18 have their aecial stage on *Abies balsamea* (L.) Mill. The material bearing the aecial stages of the remaining species is of other *Abies* species. The material of *Uredinopsis macrosperma* available to the writer was on leaves of *Abies grandis* Lindl.

The telial hosts are listed in an earlier paper by the author (8), and later in a study† of *Milesia* species.

### *The Mycelium*

The mycelium described is only for that stage of the rust borne on *Abies*. The mycelium of the 19 species, as is characteristic for the Uredinales, is intercellular, septate, uninucleate, branched, and bears haustoria in the interior of the host cells. The hyphae show some variation in diameter for the different species, and even within the species there is variation in the diameter of the mycelium. This is especially marked in the species *Hyalopsora Aspidiotus* and in *Milesia polypodophila*. In the former, the

\* Species so marked are those in which haustoria have been described in the aecial host for the first time.

† Hunter, L. M. The life histories of *Milesia Scolopendrii*, *M. Polypodii*, *M. vogesiaca*, and *M. Kriegeriana*. J. Arnold Arboretum, 17 : 26-37. 1936.

hyphae measure from  $3.4\mu$  to  $6.5\mu$ , and in the latter, when found in the leaves, from  $3\mu$  to  $4.5\mu$  in diameter. The measurements taken for the mycelium of *Pucciniastrum Epilobii* and *P. Abieti-Chamaenerii* are the same, showing a narrow range in diameter from  $2.5\mu$  to  $3.3\mu$ . The mycelium of *Melampsorella Caryophyllacearum* occurring in the leaves shows the same range in diameter as that of the two *Pucciniastrum* species. The mycelium of *Melampsora Abieti-Capraearum* may be from  $2.5\mu$  to  $4\mu$  in width. The hyphae of all the *Uredinopsis* species that occur in the Timagami Forest Reserve, and of *Uredinopsis macrosperma* from the Western States, show the same variation, namely, from  $2.5\mu$  to  $4\mu$  in diameter. More often the hyphae of all of the *Uredinopsis* species measure approximately  $3.3\mu$  in width. The mycelium of *Milesia marginalis* measures from  $2.3\mu$  to  $3.5\mu$ , but more often it is  $2.5\mu$  in diameter. The mycelium of *Milesia Kriegeriana* measures approximately the same. The hyphae of *Calypsotheca Goeppertiana* are noticeably more delicate or finer in appearance than any of the other species here considered. The measurements given for the diameter of the mycelium of this species are from  $2\mu$  to  $3\mu$ . All measurements of the mycelium of the species given above were made from hyphae that had developed freely within the air spaces of the mesophyll of the leaves.

The mycelium of *Melampsorella Caryophyllacearum* Schroet. has been described by DeBary (1) as systemic in the stem of *Abies pectinata* and causing broom formation. The mycelium of *Milesia polypodophila*, according to Faull (6), is systemic in the stem of *Abies balsamea* and causes loose broom formation. *Melampsorella Caryophyllacearum* and *Milesia polypodophila* alone, of all the species investigated by the writer, have systemic mycelium in the stem of the aecial host. The writer figures a drawing (Fig. 33) showing the mycelium of *Milesia polypodophila* as found in the intercellular spaces of the cortical tissue of infected *Abies*. The mycelium has a tendency to coil and to bear short rounded branches or knobs as well as ordinary branches. The diameter of the mycelium in the stem is from  $4.5\mu$  to  $6\mu$  in diameter, showing a higher average of width than the mycelium in the leaf. The wall of the mycelium in the leaf is less than  $1\mu$  thick, while that of the mycelium in the stem is  $1.3\mu$  thick. The age of the mycelium, in the stem examined, is at least two years. The mycelium in the leaf examined is likely not to be more than one year old, and new branches are perhaps much younger. It is interesting in this connection to recall that Sappin-Trouffy (20) found that the annual filaments in *Gymnosporangium clavariaeforme* Jacq. differed in diameter and in width of wall from the mycelium, which persisted from one year to another. The latter was found to have greater diameter and thicker walls than the former.

A table of measurements for the diameter of the mycelium of each species is given below:



Species	Diameter of mycelium in $\mu$	
	In leaves	In stem
1. <i>Melampsora Abieti-Capraearum</i>	2.5 - 4	
2. <i>Melampsorella Caryophyllacearum</i> *	2.5 - 3.3	
3. <i>Pucciniastrum Epilobii</i>	2.5 - 3.3	
4. <i>Pucciniastrum Abieti-Chamaenerii</i>	2.5 - 3.3	
5. <i>Calyptospora Goeppertiana</i>	2 - 3	
6. <i>Hyalospora Aspidiotus</i>	3.4 - 6.5	
7. <i>Milesia intermedia</i>	2.3 - 3.5	
8. <i>Milesia marginalis</i>	2.3 - 3.5	
9. <i>Milesia polypodophila</i>	3 - 4.5	4.5 - 6
10. <i>Milesia Scolopendrii</i>	3 - 4.5	
11. <i>Milesia Polypodii</i>	3	
12. <i>Milesia vogesiaca</i>	3 - 3.7	
13. <i>Milesia Kriegeriana</i>	2.3 - 3	
14. <i>Uredinopsis Atkinsonii</i>	2.5 - 4	
15. <i>Uredinopsis Phegopteridis</i>	2.5 - 4	
16. <i>Uredinopsis Osmundae</i>	2.5 - 4	
17. <i>Uredinopsis mirabilis</i>	2.5 - 4	
18. <i>Uredinopsis Struthiopteridis</i>	2.5 - 4	
19. <i>Uredinopsis macrosperma</i>	2.5 - 4	

\* DeBary (1) described the systemic mycelium of *Melampsorella Caryophyllacearum* in the stem of *Abies pectinata*.

The above table presents concisely the fact that for 17 of the species named, the diameter of the mycelium is within close range, and in many of the species the same variation is shown. Sappin-Trouffy (20) in connection with a discussion of the mycelium of the Uredinales investigated, stated that, apart from one exception, *G. clavariaeforme*, their diameter is sensibly uniform for the same species, but that it can be subject to slight modifications due to pressing in between the tissues. The writer finds modifications or variation in the diameter when the mycelium grows freely in the air spaces of the leaf or stem, as the case may be, of the host.

### The Haustoria

Haustoria are described for the species listed above. Three types of haustoria—(1) hyphal, (2) helicoid, and (3) botryose, were found. The haustorium is frequently characteristic for the species. Some variation in type within the species was found, but in no case was it so great as that described by Sappin-Trouffy (19) for *Uromyces Betae* Pers. In every case the haustorium is greatly constricted at the point where it passes through the wall into the interior of the host cell. Invagination of the protoplasmic membrane seems to be the type of invasion. All the haustoria examined are uninucleate. No instances could be positively ascertained where more than one nucleus occurred in the usual nonseptate haustorium. In connection with the nucleus of the haustorium of *Milesia marginalis*, very occasionally, the writer found some extrachromatin material lying in the cytoplasm nearby the haustorial nucleus. In a few cases sheaths were found to be present around the haustoria. This was true for a few species only. Moss (16)

found sheaths or partial sheaths around the haustoria in the telial hosts of the following species—*Hyalopsora Aspidiotus*, *Milesia marginalis*, *Milesia polypodophila*, and *Uredinopsis Osmundae*. He also found branching a much more common feature of the haustoria in the telial hosts than the writer finds in the haustoria in the aecial hosts. A number of species under consideration have haustoria that bear a close relation to the host nuclei. Host nuclei are often indented by branches of the haustoria. Host cells invaded by haustoria are frequently filled with vacuolate cytoplasm, a condition that is common in the healthy leaves of *Abies balsamea*. Chloroplasts and starch grains are frequently met with in the invaded cells as in those free from the rust. Perhaps these conditions are to be expected since the *Abies*' hosts are all highly susceptible to the rusts borne on them, and a symbiotic relation (which exists in the early condition of the infection) causes less disturbance in the host than would happen if the hosts showed resistance to infection. In most of the material examined, the rust is comparatively young. Much of the material bears spermogonia in incipient stages, or mature spermogonia showing aecia in incipient stages. Even when leaves bearing mature aecia were examined for haustoria, sheaths, as a rule, were not commonly found. Ward (22) states: "In all cases where haustoria are developed, the mycelium enters into a peculiar symbiotic connection with the cells and for some time taxes them, as it were, rather than injures them directly". Rice (18) in the discussion of the haustoria of certain Uredinales concludes that they do little damage to the host except at the time of spore production. In connection with *P. Sorghi*, Mains (15) states that no harmful effect is shown by the host until after some period of time.

A description of the type of haustorium peculiar to each of the species studied follows:

*Melampsora Abieti-Capraearum* (Figs. 1 to 6)

The haustoria of *M. Abieti-Capraearum* are hyphal, simple or branched freely. They are uninucleate and are very frequently found in close association with the host nucleus, sometimes making a curve almost completely around it. Fig. 2 shows a haustorium that is septate. This is one of only two instances of septation found in the haustorium of any of the species investigated by the writer. Fig. 3 shows a young haustorium that is hyphal, very regular in outline, and surrounded by the cytoplasm of the host cell. The smaller, lower cell in Fig. 2 shows the narrow passage of the haustorium through the host cell wall. The mycelium just outside of the cell wall shows some indication of swelling but cannot properly be called an appressorium. Fig. 6 shows a much branched haustorium with one of the branches coming off from the side of the main branch. The haustoria are the same width as the intercellular mycelium or slightly dilated. Two or three haustoria may be present in a host cell, but it is more usual to find only one. The haustoria of this species occur frequently and are found in the mesophyll and in the epidermal cells of the leaf. Sheaths are sometimes present around the haustoria. None are figured here. As well as on *Abies balsamea*, haustoria

were found in dried herbarium specimens of *M. Abieti-Capraearum* on *Abies pectinata* D.C. Haustoria of a well developed hyphal type occur in the mesophyll cells of the leaf. Mature haustoria appear to be sheathed. No branching of haustoria was observed.

*Melampsorella Caryophyllacearum* (Figs. 7 to 14)

The botryose haustoria of *Melampsorella Caryophyllacearum* (*M. Cerastii* (Pers.) Schroet.) (1) were described first by DeBary (1) for the aecidial host. Pady (17) reports that on *Abies lasiocarpa* the haustoria differ in shape and size with a knoblike or irregular swelling at the tip from which numerous short fingerlike projections extend. Haustoria of a very complicated nature were reported by Magnus (11) on the mycelium of the uredinial host. Moss (16) described and figured botryose haustoria in the telial host. Schroeter (21) had already, in 1874, described the mycelium of the rust in the uredinial stage but was unable to locate haustoria. The writer has been able to find a number of stages in the development of the haustoria in the leaves of young diseased buds of *Abies balsamea*. Fig. 7 shows a branch pressing the cell wall inward. This is similar to DeBary's (1) findings for the same species in *Abies pectinata*. The figures from 7 to 13 represent a progressive development in the growth of the haustorium. The young haustoria are hyphal-like and slightly irregular, but very soon show indications of swelling and lobing. The mature haustorium is dilated and very much lobed (Fig. 14) in appearance. Fig. 12 shows a lobed haustorium attached to an appressorium by means of a very narrow passageway through the wall of the host cell. The haustorium in the outer cell at the right in Fig. 8 shows a connection with a slight appressorium. Figs. 7, 9, and 10 show either very slight or no appressoria. The haustoria are uninucleate and are frequently found pressing tightly against the host nuclei (Figs. 8, 11, 13, and 14). In all cases the haustoria are surrounded by the cytoplasm of the host cell. Chloroplasts are shown to be present in some of the cells (Fig. 8). Small starch grains are fairly abundant (Figs. 10 to 12). The haustoria occur abundantly in the mesophyll and in the epidermal cells, and occasionally even invade the vascular bundle. A mature haustorium in the spongy mesophyll of the leaf is found to be uninucleate and four-lobed. With a very high magnification the lobes appear to be indented giving a botryose appearance. In the vascular tissue of the leaf within a large woody cell two small uninucleate curved haustoria occur. This woody cell is in the transfusion tissue within the epidermis. It is frequently observed in longitudinal sections of the leaf that the mycelium runs for long distances in close association with the phloem.

*Pucciniastrum Epilobii* (Fig. 15)

The haustorium of *P. Epilobii* may be simple, hyphal, fairly straight, or curved. Frequently it is bulbous for about one-third of its length immediately after entering the host cell, as shown in Fig. 15. Occasionally the haustorium is branched. It is surrounded by the cytoplasm of the host cell. The connection between the haustorium and the intercellular mycelium is

shown in Fig. 15. No appressorium is shown here although appressoria are commonly found. The haustorium is only occasionally in contact with the nucleus of the host cell. Starch grains and chloroplasts appear normally in the cells where haustoria are found. No entire sheaths were located in sections of leaves that bore pycnia only, but occasionally small red staining areas (where safranin was used) were found lying close to the wall of the haustorium indicating the beginning of sheath formation. In the writer's opinion the haustoria of this species occur less frequently than in any of the other rusts on *Abies balsamea*. Nevertheless they have a greater variety of location and may be found in the epidermal cells, in the mesophyll cells, in the larger cells of the resin canals, and in the thin-walled cells of the pericycle of the leaf. Leaves that bore very mature aecia were examined for haustoria. It is interesting to note that complete sheathing of the haustoria was more commonly found in this material. When the haustorium was completely sheathed the cytoplasm of the host cell was greatly reduced. The writer's findings for this species would seem to be in agreement with Colley's (3) suggestion that, as in *Cronartium ribicola*, the development of the sheath seems to come with maturity. Moss (16) described allantoid or irregularly cylindrical haustoria for the mycelium of the uredinal stage of *P. pustulatum* (Pers.) Diet., and also for *P. americanum* (Farl.) Arth. He found that the haustoria are frequently in contact with the host nuclei and that sheaths are commonly developed around the haustoria.

*Pucciniastrum Abieti-Chamaenerii*

The form of the haustorium of *P. Abieti-Chamaenerii* is in general the same as that of *P. Epilobii*, excepting that the writer does not find branching in the former. No indications of sheathing were found in the haustoria of this species but the material examined was young and no leaves bearing aecia were investigated. The haustoria as in *P. Epilobii* are rarer than for the other species of rusts on *Abies balsamea* and they also have the same range of distribution in the host cells as is found in *P. Epilobii*.

*Calypsotheca Goeppertiana* (Figs. 16 to 18)

The haustorium of *C. Goeppertiana* is hyphal-like. It is of the same width or slightly wider than the intercellular mycelium and is frequently irregular in outline. No appressoria of any appreciable size were observed. The mycelium is much constricted in its passageway through the wall of the host cell, but immediately after entering the host cell the haustorium becomes very slightly bulbous (Figs. 16, 17). Fig. 18 is not a median section through the haustorium. The haustoria are frequently found in close association with the nuclei of the host cells (Figs. 16, 18). The haustoria are uninucleate and are surrounded by cytoplasm. The haustoria occur quite abundantly in the mesophyll tissue of the leaf. Hartig (7) illustrates saclike haustoria of this rust in *Vaccinium*. The writer, too, has observed short, stout haustoria in *Vaccinium* and finds that those in the *Abies* host are slender, hyphal, and longer than those in the telial host.

*Hyalopsora Aspidiotus* (Figs. 19 to 21)

The haustorium of this species is hyphal in nature. It is frequently curved or sharply bent (Fig. 21), but may lie within the host cell as a straight clavate organ. Branching of the haustorium occurs sometimes. When it does occur it is never profuse. An appressorium is formed on the intercellular mycelium, previous to the entrance of the haustorium into the host cell. There is a marked constriction of the mycelium at the point of entrance. The mature haustorium is slightly dilated so that it is of somewhat greater diameter than the intercellular mycelium (Fig. 19). The haustorium is uninucleate. The haustoria are quite distinctive on account of their size. They are found occasionally encircling the host nucleus (Fig. 20) and sometimes indent it. Fig. 20 shows a haustorium in a cell in which chloroplasts occur normally. Starch grains and chloroplasts usually occur normally in all the cells of the mesophyll tissue in which haustoria are found. Fig. 19 represents an invaded cell showing only cytoplasm as appearing in that particular section. The haustoria are very abundant. They are found in the cells of the epidermis and in the mesophyll tissue of the leaves. Magnus (9) reported haustoria for *Hyalopsora Polypodii* in its telial host in 1892. In 1895 Magnus (10) stated that haustoria were not borne on the intercellular mycelium of *H. Aspidiotus*. Moss (16), in 1926, announced the occurrence of haustoria for this species in the telial host and described them as clavate or allantoid in shape and occasionally slightly lobed. He also found that sheaths were rarely present around the haustoria. The writer reports the finding of haustoria of *Hyalopsora Polypodii-Dryopteridis* in the aecial host, *Abies pectinata*, of European material obtained from Dr. Eugène Mayor. The haustoria resemble those of *H. Aspidiotus* and are of the clavate type. No sheathing of haustoria was found in either species.

*Milesia intermedia* (Figs. 22, 23)

The haustorium of *M. intermedia* is slender and hyphal-like, regular or irregular in outline, unbranched or occasionally showing a tendency to branch (Figs. 22, 23). The haustorium may be curved at the distal end. The mycelium immediately outside the constricted passage of the haustorium into the host cell is slightly swollen in the form of an appressorium (Fig. 22). The uninucleate haustorium (Fig. 22) is somewhat dilated and is in close association with the host nucleus. This contact is of fairly frequent occurrence. The cytoplasm of the host cell appears normal and chloroplasts occur. The cell at the left in Fig. 23 shows a young haustorium of clavate form. In both cells in this figure the cytoplasm is slightly drawn away from the wall of the host cell. The haustoria are abundant in occurrence. They are found in the cells of the epidermis, in the mesophyll tissue, and in the thin-walled cells of the pericycle. The mycelium separates the cells of the endodermis and invades the cells of the pericycle lying immediately within. Magnus (12) was unable to find haustoria for *M. Kriegeriana* in the telial host. He (13) also found no haustoria for *M. Feurichii*, but he did find short knoblike

swellings in the intercellular mycelium. Haustoria have not hitherto been reported for either the aecial or telial stage of *Milesia intermedia*.

*Milesia marginalis* (Figs. 24 to 26)

The haustorium of this species is slender and hyphal in character. The form may vary from slender, fairly straight hyphal haustoria to twisted, lobed, or branched haustoria. The haustoria are often curved at the tip. They are uninucleate. One case of septation of the haustorium was observed, as in *Melampsora Abieti-Capraearum*. In each cell of this unusual haustorium of *Milesia marginalis* a nucleus was observed. Septation occurred at about one-quarter of the length of the haustorium from its distal end. Fig. 26 shows the constricted passage of a haustorium through the host cell wall. No appressoria are shown in the figures but they normally occur. Starch grains and chloroplasts are found in the invaded cells, which are frequently occupied by vacuolate cytoplasm. Both cells of Fig. 25 show digestive areas around the haustoria. The dark line around the clear space surrounding the haustorium does not represent a sheath, but is merely the writer's method of calling attention to the digestive vacuole. Fig. 24 shows the beginning of a digestive vacuole. Sheathing of the haustorium in *M. marginalis* was found in only one instance. The haustoria are abundant. They are found in the cells of the epidermis, in the mesophyll tissue, and in the thin-walled cells of the pericycle, lying immediately within the endodermis. The haustoria may occur in close association with the host nucleus (Fig. 24), but more frequently they are found lying in the cell quite free from it (Figs. 25, 26). Occasionally the host nucleus is found indented by an arm of the haustorium (Fig. 24). Moss (16), in 1926, described the haustoria for the telial host. He (16) figures them as much branched organs of stouter nature than those represented by the writer as occurring on the aecial host of *M. marginalis*. He also observed sheaths of cuplike thickenings at the constricted bases of the haustoria.

*Milesia polypodophila* (Figs. 27 to 37)

The mycelium of this interesting species is systemic in the stem of the aecial host. According to Bell (2) and Faull (6) loose brooms are formed in the infected areas. The material used for examination was pieces of infected stem of *Abies balsamea* with attached leaves. Haustoria were found in both leaf and stem. They occur in the mesophyll cells of the leaf and in the cells of the cortex of the stem. The mature haustorium is spiral or helicoid in nature. Sometimes it is a true spiral and in the stem it may have as many as seven coils (Fig. 35). Quite frequently it is more loosely arranged and has four or five coils (Fig. 36). The haustoria found in the stem, almost without exception, were of the large coiled type (Figs. 34 to 37). The haustoria in the leaf are of the spiral type too. Figs. 27 to 32 are all of cells from a young invaded leaf. Fig. 28 shows a part of a haustorium that has at least three coils. All the haustoria are uninucleate and are frequently found in close association with the host nuclei. The haustoria in the leaf (Figs. 27 to 32)

have not as heavy walls as those found in the stem (Figs. 34 to 37). Fig. 29 shows a young haustorium in which the distal end is beginning to curve. Fig. 30 shows one somewhat more advanced developing a loose coil. Fig. 31 shows a haustorium that has developed a closer spiral. In Figs. 30 and 31 are shown the constricted passages of the mycelium through the cell wall, the slightly enlarged mycelium forming appressoria, and the coiled nature of the mycelium in the intercellular leaf spaces. In Fig. 27 the cells at the left abut on the stem. The mycelium is also closely projected against the stem. More intercellular mycelium is found closely applied to a nearby cell (Fig. 28). Fig. 29 represents a cell a little farther up the leaf, away from the stem, and Fig. 31 one still farther on, indicating the more or less complete invasion of the leaf by the parasite. The cytoplasm, chloroplasts, and starch grains appear to be normal in the invaded cells. Fig. 36 shows a haustorium in a cell of the cortex of the stem. Except for the presence of the invading organ, the cell is normal in appearance. Starch grains lie in the cytoplasm and the haustorium, which is in contact with the host nucleus, frequently borders on the large vacuole (Fig. 36). The writer was unable to trace the cytoplasmic layer all the way around the haustoria (Figs. 34, 36), but the cell contents appear so normal that it is likely that a very thin cytoplasmic membrane does persist around the haustoria. Fig. 37 shows a cell of the cortex in which the host cytoplasm seems to be breaking down. The host nucleus is not in contact with the haustorium. The nucleus of the haustorium itself shows a nucleolus, and appears to be in the resting stage. Figs. 34 to 36 show appressoria. Sheaths were not found to be present around the haustoria. The haustoria of *M. polypodophila* are numerous. As many as four have been found in a cell of the stem. Moss (16) describes a much branched haustorium for this species in its telial host. From his figures it is concluded that the haustorium in the telial host is very different in form from that in the aecial host. Dodge (4) calls attention to the different types of haustoria of *Gymnosporangium clavipes* Cke. & Pk. In the aecial host the haustorium is hyphal, long, irregular, and coiling, while in the telial host it is regular in outline and trim. Evans (5) also states that the haustoria of certain *Puccinia* species are very distinctive.

#### *Milesia Scolopendrii* (Fig. 41)

H Haustoria of *M. Scolopendrii* occur on *Abies alba* Mill. and also on *A. concolor* Lindl. & Gord. in the mesophyll and epidermal cells of the infected leaves. The haustoria frequently have a slightly suggestive spiral (Fig. 41), or a very loose spiral form or, on the other hand, they may be merely hyphal. They are found in contact with the host nucleus (Fig. 41) or lying in the cytoplasm free from it. They are uninucleate, with vacuolate cytoplasm. They are more commonly not branched but may be branched dichotomously. Occasionally haustoria are observed with small knobs that may be the beginning of branches. The haustorium is connected to the intercellular mycelium by a very narrowly constricted passage through the cell wall. There is evidence that the leaf tissue of *A. concolor* is much more adversely

affected by the rust invasion than that of *A. alba*. The haustoria in epidermal cells are shorter than those in the mesophyll cells. They are hyphal, usually inflated at the base, and narrowed toward the tip or distal end.

*Milesia Polypodii* (Figs. 42 to 43)

Haustroria of *M. Polypodii* are found in mesophyll and epidermal cells of leaves of *Abies concolor* and *A. alba* infected with the rust (O, I). The haustoria are hyphal (Fig. 42) but often irregular or branched dichotomously or branched much more freely (Fig. 43). A lesser lobed or knobbed effect is often observed. The distal end of the haustorium is frequently found in contact with the host nucleus. As many as three branches have been found indenting the nucleus. The haustorium itself is uninucleate and has vacuolate cytoplasm. In a section from which Fig. 43 was drawn, while observed under oil immersion, the proximal end of the haustorium could be viewed connected by a narrowly constricted passage through the cell wall and a somewhat inflated appressorium beneath it. The haustoria of *M. Polypodii* are characteristic and serve as an aid in identification of the species. The host cells of *Abies concolor* are often to some extent affected adversely in comparison with those of *Abies alba* infected with *Milesia Scolopendrii*. Figs. 42 and 43 represent cells that, apart from the invasion by haustoria, appear to be normal.

*Milesia vogesiaca* (Fig. 44)

Haustroria occur in the mesophyll and epidermal cells of infected leaves of *Abies alba*. They are uninucleate, containing vacuolate cytoplasm, and are mainly hyphal in nature. Occasionally some are found that have a suggestion of a very loose spiral form. Branching is rare. The distal end of the haustorium is frequently in contact with the host nucleus (Fig. 44). Haustroria are embedded in the cytoplasm of the host cell. In the epidermal cells the haustoria are hyphal but a few were found with somewhat inflated tips. The haustoria enter the cell through a constricted passage in the wall. When the rust is more mature there is evidence of sheathing of the haustorium.

*Milesia Kriegeriana* (Fig. 45)

Haustroria of *M. Kriegeriana* occur in the epidermal and mesophyll cells of the infected leaves of *Abies alba*. The haustoria are uninucleate with vacuolate cytoplasm and hyphal in form (Fig. 45) lying in the cytoplasm of the host cell. Frequently the tip of the haustorium is in contact with the host nucleus. The haustoria of the epidermal cells are shorter than those of the mesophyll cells. They are hyphal and inflated slightly at the basal end. The haustoria of this species resemble those of *Milesia intermedia* on *Abies balsamea*, and are more distinct or characteristically one type than are the haustoria of *M. Scolopendrii* or *M. Polypodii*.

*Uredinopsis Atkinsonii* (Figs. 38 to 40)

The haustorium of *U. Atkinsonii* is typically hyphal in character and unbranched. It is slightly wider in diameter than the intercellular mycelium



and is uninucleate. The wall of the haustorium is irregular. The appressorium is very slight. Chloroplasts and starch grains are found in the cells invaded by the parasite (Figs. 39, 40). The protoplasm is pushed in by the mycelium as it grows. Colley (3) is certain that the haustoria of *Cronartium ribicola* Fischer do not pierce the plasma membrane of the host. Figs. 38 and 40 show the narrow passage through the host cell wall by which means the very much constricted mycelium enters the host cell. The writer occasionally observed a haustorium lying closely alongside the host nucleus, but not indenting or deforming it. The haustoria examined were from leaves that bore mature or incipient stages of spermogonia. The haustoria are fairly abundant. They occur in the leaf in the epidermal cells, and in both the thin and heavy walled cells of the pericycle, though rarely in the latter. The intercellular mycelium separates the cells of the endodermis and passes in between them towards the pericycle. As noted previously Magnus (9), in 1892 and again (14) in 1904, has stated that there are no haustoria in the genus *Uredinopsis*. Moss (16), in 1926, described lobed and branched haustoria, in the telial hosts, for three *Uredinopsis* species, namely, *U. Atkinsonii*, *U. Osmundae*, and *U. Phegopteridis*. Moss (16) figures haustoria for *U. Atkinsonii* and *U. Osmundae*. These figures show much stouter haustoria than the writer finds on the mycelium of *U. Atkinsonii* in the aecial host. Also branching of the haustorium was not found by the writer in the aecial host. Moss (16) described sheaths for the mature haustoria in the telial hosts. The writer finds no sheaths in the material examined for the haustoria in the aecial host.

#### *Uredinopsis* Species

Haustoria have been found by the writer in the *Uredinopsis* species on *Abies balsamea*, namely *U. Atkinsonii*, *U. Phegopteridis*, *U. Osmundae*, *U. mirabilis*, and *U. Struthiopteridis*. In each species they are hyphal in form and are very similar in type. Slight appressoria usually occur just outside of the host cell. From the appressorium a narrow tube penetrates the host cell wall. No sheathing of the haustoria was observed. Even in the dried herbarium material of *Uredinopsis macrosperma* remains of hyphal-like haustoria were observed in the epidermal and mesophyll cells of the leaf.

#### Conclusion

Further study of more mature aecial material of most of the species investigated should prove interesting. Such an investigation would finally determine whether the haustoria, at maturity of the rust, are frequently sheathed, and also to what extent, and perhaps in what way, the host cells are injured. Many of the rusts of *Abies* that develop pycnia or spermogonia and aecia on leaves of the current season cause blanching of the infected leaves and early defoliation. This is very true for *Abies balsamea* when infected with *Uredinopsis* species. According to Bell (2) the needles infected with *Milesia polypodophila* turn pale green, but are never blanched as those infected with

*Uredinopsis* species. Leaves bearing only spermogonia or both spermogonia and aecia of *M. polypodophila* or *Hyalopsora Aspidiotus* show stimulation by the parasite. The infected leaves appear somewhat enlarged even in the late pycnial stage. There is also a difference in the staining reaction of the host tissue as compared with the normal leaf of *Abies balsamea*.

This investigation, initiated at the University of Toronto, has been carried on at intervals since. The writer wishes to thank Dr. J. H. Faull of Harvard University for the abundance of excellent material offered for the investigation, and also Dr. Eugène Mayor, Switzerland. Thanks are extended to Dr. Faull for helpful criticism at intervals during the investigation.

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(Note: Figs. 1-45 will be found on pp. 235-238. All figures are camera lucida drawings made by the author.)

### Explanation of Figures

FIGS. 1-6. *Haustoria of Melampsora Abieti-Capraearum in the mesophyll cells of the leaf of Abies balsamea.*  $\times 518$ .

FIGS. 7-13. *Haustoria of Melampsorella Caryophyllacearum in young and developing stages in the cells of the mesophyll of the leaf of Abies balsamea.*  $\times 518$ .

FIG. 14. *A mature haustorium of M. Caryophyllacearum in a cell of the mesophyll of the leaf of Abies balsamea.*  $\times 464$ .

FIG. 15. *The haustorium of Pucciniastrum Epilobii in a cell in the mesophyll of the leaf of Abies balsamea.*  $\times 643$ .

FIGS. 16-18. *Haustoria of Calyptospora Goeppertiana in the mesophyll cells of the leaf of Abies balsamea.*  $\times 518$ .

FIGS. 19-21. *Haustoria of Hyalopsora Aspidiotus in the mesophyll cells of the leaf of Abies balsamea.*  $\times 555$ .

FIGS. 22-23. *Haustoria of Milesia intermedia in the mesophyll cells of the leaf of Abies balsamea.*  $\times 518$ .

FIGS. 24-26. *Haustoria of Milesia marginalis in the mesophyll cells of the leaf of Abies balsamea.*  $\times 518$ .

FIGS. 27-32. *Haustoria of Milesia polypodophila in the mesophyll cells of the leaf of Abies balsamea.*  $\times 560$ .

FIG. 33. *The intercellular mycelium of M. polypodophila in the cortex of the stem of Abies balsamea.*  $\times 500$ .

FIGS. 34-37. *Haustoria of M. polypodophila in the cells of the cortex of the stem of Abies balsamea.* FIGS. 35 and 37,  $\times 560$ ; FIGS. 34 and 36,  $\times 600$ .

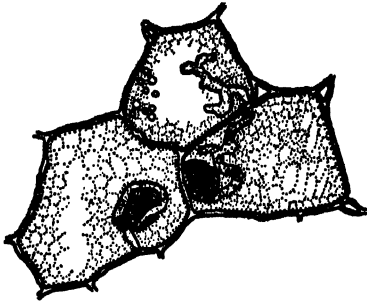
FIGS. 38-40. *Haustoria of Uredinopsis Atkinsonii in the mesophyll cells of the leaf of Abies balsamea.*  $\times 518$ .

FIG. 41. *Haustorium of Milesia Scolopendrii in the mesophyll of the leaf of Abies alba.*  $\times 740$ .

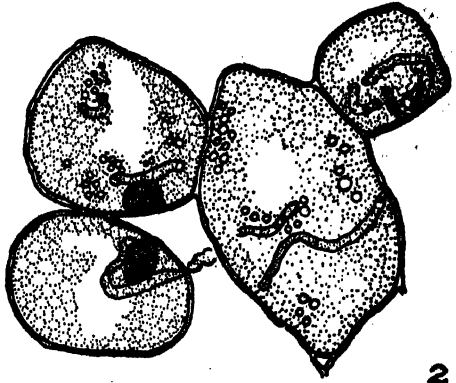
FIGS. 42-43. *Haustoria of Milesia Polypodii in the mesophyll of the leaf of Abies concolor.*  $\times 740$ .

FIG. 44. *Haustorium of Milesia vogesiaca in the mesophyll of the leaf of Abies alba.*  $\times 740$ .

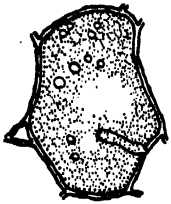
FIG. 45. *Haustorium of Milesia Kriegeriana in the mesophyll of the leaf of Abies alba.*  $\times 740$ .



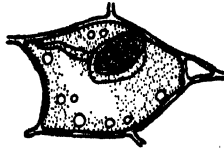
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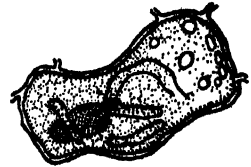
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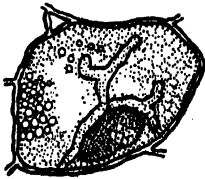
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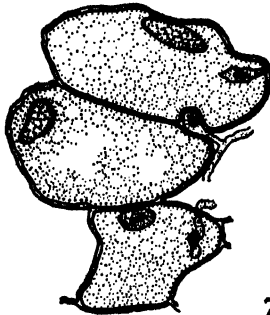
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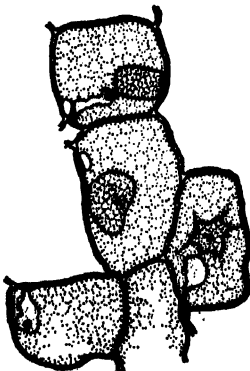
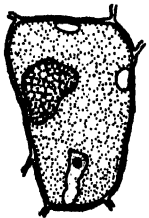
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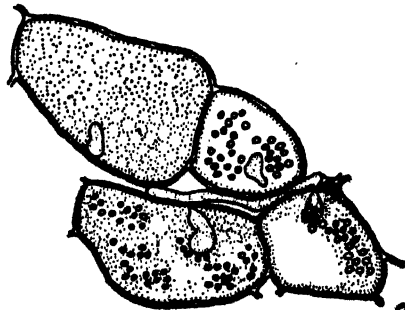
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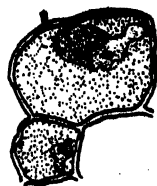
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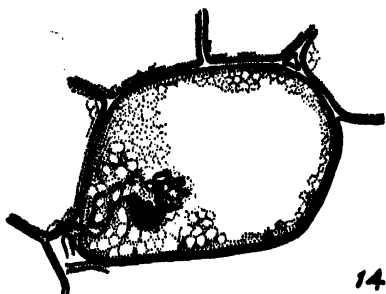
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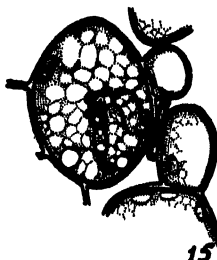
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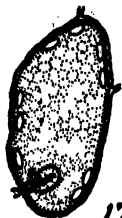
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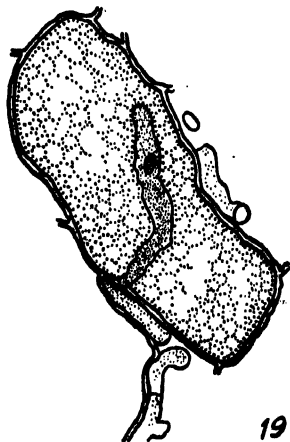
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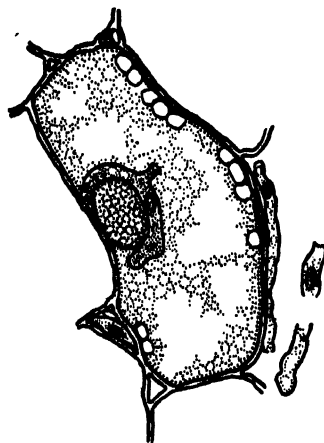
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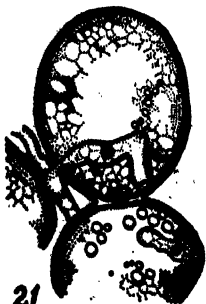
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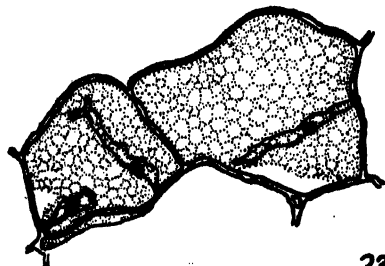
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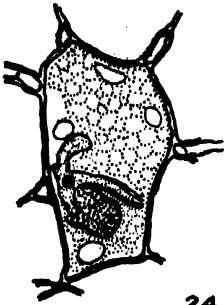
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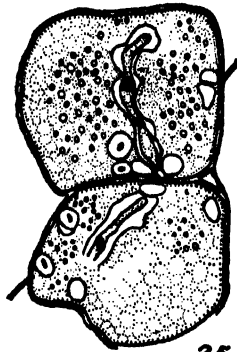
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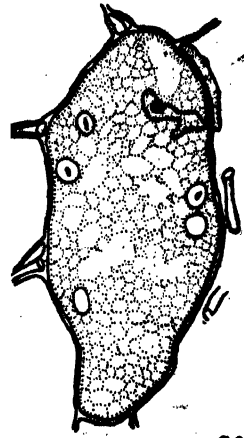
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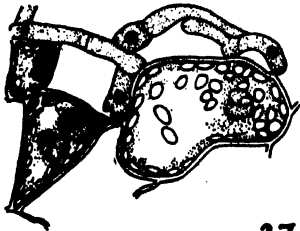
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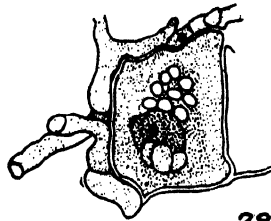
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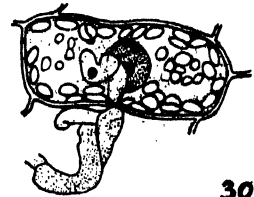
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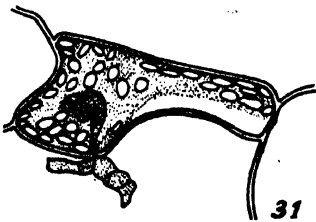
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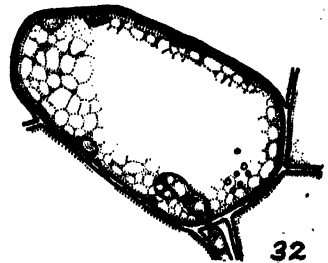
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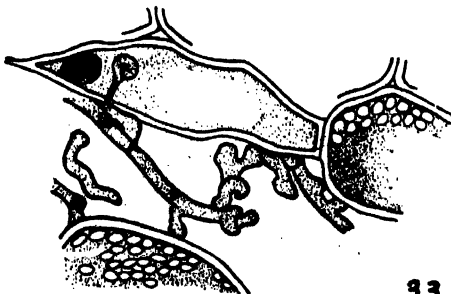
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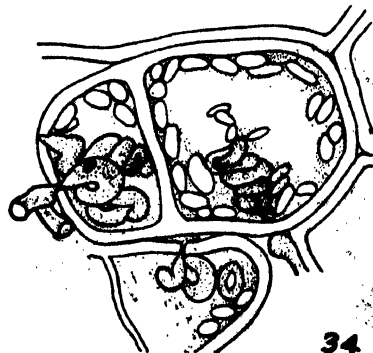
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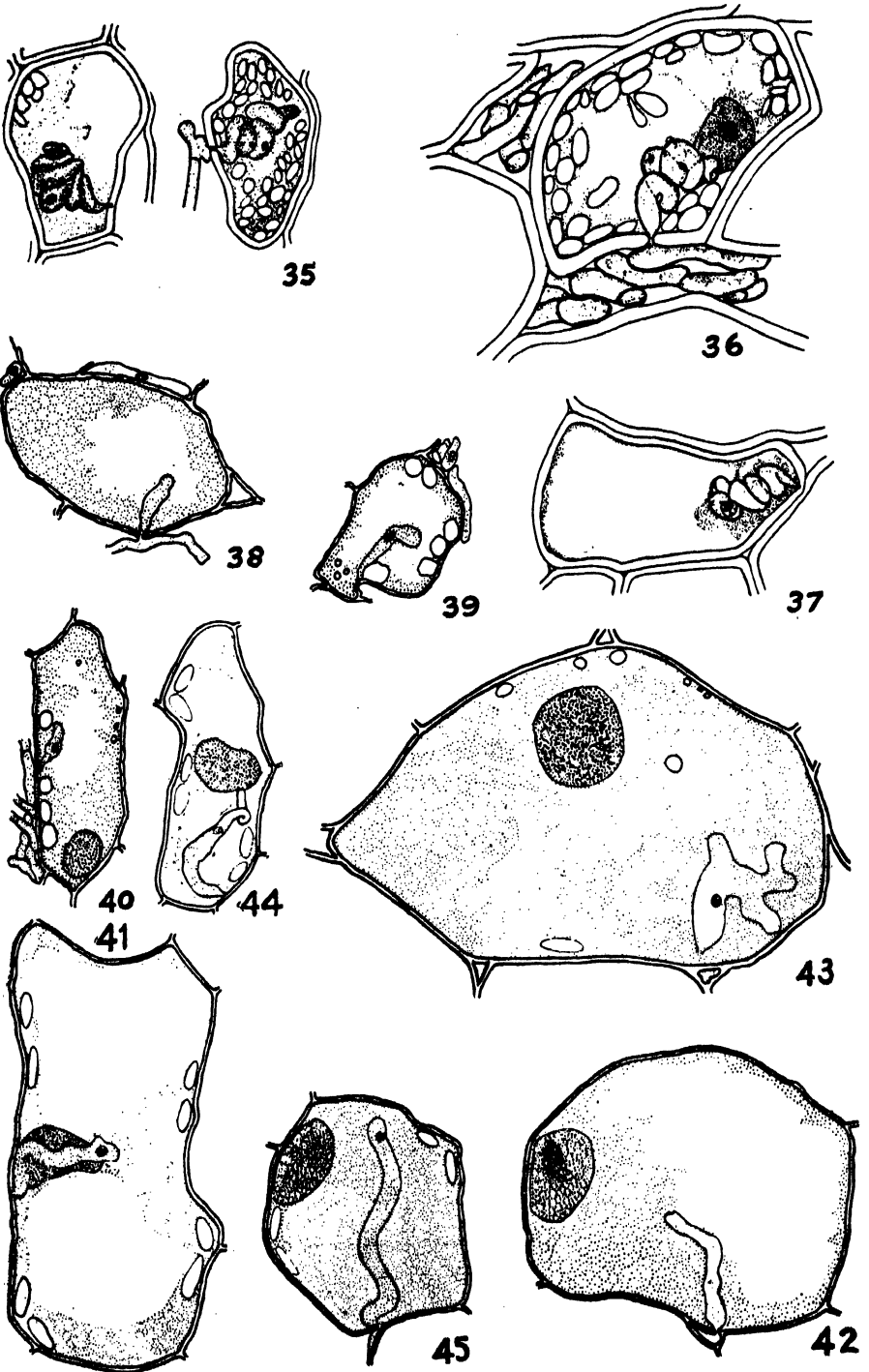
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## TRANSLOCATION OF 2,4-D IN INJECTED FLOWERING PERENNIAL SOW THISTLE PLANTS<sup>1</sup>

By W. G. CORNS<sup>2</sup>

### Abstract

Visible response to injection of flower heads of perennial sow thistle plants with herbicidal concentrations of 2,4-D appeared only in the uppermost regions of the plants. The herbicide moved downward through peduncles supporting treated heads, then it moved upward through neighboring peduncles. Apparently this pronounced ascent of herbicide was associated with transport of plant solutions to regions of rapid development and transpiration.

On the other hand, response to injection of severed stems of flowering sow thistle plants was not limited to the region of treatment. The herbicide moved downward in treated stems, upward in their intact lateral branches, leaves, and flowers, and extensively downward and laterally in the root system.

Significance of this multidirectional movement is discussed.

### Introduction

Translocation of chemical from the tops down through the extensive root system of perennial weeds is obviously of first importance in effecting their eradication or control by means of herbicides. It is relatively easy to kill the aerial growth of such plants but it is often extremely difficult to bring about adequate penetration of chemical into underground parts that are so effective as a means of reproduction. There is, therefore, a need for further studies, under natural conditions, of factors affecting the transport of herbicides in various plants.

The present paper reports a study of the movement and effects of the selective weed killer, 2,4-dichlorophenoxyacetic acid (or reaction product) following its application to flowers and to severed stems of perennial sow thistle plants (*Sonchus arvensis*) growing under field conditions in black loam soil at Edmonton.

Access to literature relating to natural and synthetic growth regulating substances is available in the publications of Boysen Jensen (3), van Overbeek (10), Thompson *et al.* (12), Thomson (13), Went (16), Zimmerman and Hitchcock (17), and others.

It is generally agreed that movement of growth regulators from leaves into other parts of plants is associated with the translocation of organic food material mainly in the phloem and parenchyma cells. Hitchcock and Zimmerman (5) and Ferri (4) showed that synthetic growth substances can move upward in plants from the soil or from application in lanolin around the stem. Hitchcock and Zimmerman (6) also report that applied growth substances such as indoleacetic and indolebutyric acids apparently move up and down in

<sup>1</sup> Manuscript received February 4, 1948.

Contribution from the Department of Plant Science, University of Alberta, Edmonton, Alta.

<sup>2</sup> Assistant Professor.



plant tissues. Beal (2) demonstrated downward movement of 4-chlorophenoxyacetic acid to roots from treated stems of sweet pea plants. Weaver and DeRose (15) in their studies of translocation of 2,4-D in young bean plants also obtained evidence to support the theory of upward movement of growth regulators in the xylem and downward movement mainly in the phloem. Mitchell and Brown (7) working with bean plants observed that 2,4-D applied to leaves having a low sugar content, or to young rapidly growing leaves, produced little or no visible response in other parts of the plants. Under conditions favorable to translocation of organic food materials, the reactant moved into the stems but apparently did not move upward through the petioles of opposite leaves. Thus, as one might expect, the extent and direction of transport of 2,4-D or its reaction products are shown not to be completely independent of other translocation processes within the plant.

Published reports of basic studies of translocation of 2,4-D in deep rooted perennial weeds are relatively few in number. Tukey and Hamner and their associates have described histological changes in sow thistle and bindweed (14), and biochemical changes in the latter (11), following application of 2,4-D. They found definite similarities in the general mobilization of carbohydrates and nitrogen of bindweed as compared with the effect of other growth regulators on bean seedlings (11). This provides some indication that results with annual plants may have general application in connection with effects of translocation of 2,4-D.

Apparently the application of 2,4-D specifically to flowers and peduncles of plants such as tomato and cucumber has usually been done with concentrations less than 100 p.p.m. in experiments designed to study production of seedless fruits (17). It has already been noted that in the present work herbicidal concentrations of 2,4-D were applied to flowers and cut stems of perennial sow thistle. The work of Arny (1) shows that readily available carbohydrates in roots of sow thistle plants are least at flowering time. Hence we might expect that treatment at this stage of growth would be timely for purposes of weed control. Field practice seems to support this view (9).

## Experimental

### 1. INJECTION OF FLOWER HEADS OF PERENNIAL SOW THISTLE

#### *Experiment I*

##### *Materials and Methods*

Preliminary tests were performed on sow thistle plants growing in a heavily infested garden. Rows of plants approximately three feet apart were used after hoeing out the unwanted plants.

At the early flowering stage a single head on each plant was tagged for treatment (July 17). An 0.1 ml. aliquot of 2,4-D solution applied from a graduated 1 ml. hypodermic syringe was carefully delivered, without runoff, among the bases of the flowers making up an individual head. Rows of 15 plants each were treated as follows:

- (1). Check—no treatment.
- (2). Check—distilled water only.
- (3). Triethanolamine formulation of 2,4-D (Naugatuck brand).
- (4). Sodium salt of 2,4-D (J. T. Baker Co.).
- (5). Morpholine salt of 2,4-D (Baker).

Each formulation was used to make 0.5, 1, and 2% solutions calculated as 2,4-D acid. A row of plants was treated with each solution, 0.1 ml. being applied as previously indicated to one flower head per plant. All the heads on each plant were then covered with a glassine bag to prevent seed dispersal. A week later bags were removed temporarily during observations of effects of treatment.

### Results

Counts of total numbers of dead heads and dead peduncles on 10 plants per row appear in Table I.

TABLE I

TOTAL NUMBER OF AFFECTED PARTS ON 10 SOW THISTLE  
PLANTS ONE WEEK AFTER TREATMENT OF ONE FLOWER  
HEAD PER PLANT WITH 0.1 ML. 2,4-D SOLUTION

2,4-D formulation	Dead flower heads	Dead peduncles
Triethanolamine		
0.5%	10	5
1.0%	10	7
2.0%	19	19
Sodium		
0.5%	10	4
1.0%	16	13
2.0%	22	22
Morpholine		
0.5%	10	1
1.0%	13	13
2.0%	40	40

### Checks

Some flower heads were closed and dry with yellowing peduncles but none had blackened involucre or withered peduncle

Flowers killed by 2,4-D were distinguished by their blackened brittle involucre from those closed and dry as a result of other causes. Dead peduncles were those visibly burned and withered by the chemical, which obviously moved downwards from the point of treatment and in many cases then moved up neighboring peduncles killing them and their flowers. This behavior is consistently borne out by the data, which show increased burning response to 2,4-D transport resulting from increasing concentration of chemical. Because of displacement of flower stalks caused by enclosure in bags, it was

not possible in this test to distinguish peduncles not killed but showing bending response attributable to the 2,4-D treatment.

Apparently as a result of the confinement of the flower heads within the bags used to prevent seed dispersal, virtually all flowers on treated and untreated plants became sterile. All flower heads treated with 2,4-D were killed but obviously it was impossible to reach any conclusions as to the possible effects of the chemical on yield and viability of seed of untreated flowers on treated plants. Unfortunately for purposes of comparison there was no unbagged material in the same experimental area. Examination of a bulked sample of seed collected from plants in other locations, however, indicated that under natural conditions at least 40 or 50% seed set can be expected.

### *Experiment II*

#### *Materials and Methods*

Plants for this experiment and the one to be described in Expt. III (Injection of cut stems) were grown from pieces of root stock about  $\frac{1}{4}$  in. in diameter and 2 in. long, transplanted in June with spacing three feet apart within and between rows. Aqueous solutions of morpholine salt of 2,4-D (Baker) were used in 1%, 2%, and 4% concentrations (as 2,4-D acid) and 0.1 ml. was applied per plant as in Expt. I. Treatments were randomized, a group of 15 plants receiving each concentration of solution on Aug. 6 (early flowering stage, plants about 26 in. tall). Water treated and untreated controls were included.

In this experiment inflorescences were not bagged until a week after treatment, hence it was also possible to note the number of peduncles noticeably bent or twisted, but not withered as a result of movement of herbicide.

#### *Results*

Evidence of penetration of growth regulator was visible within four or five hours from time of treatment. Peduncles of several treated flower heads became limp and darkened in color during this time. A summary of results appears in Fig. 1.

Comparison of results shown in Table I with those given in Fig. 1 indicates somewhat less injury per plant in Expt. II. This may be mainly attributable to the fact that inflorescences were not covered immediately after treatment of plants in the second test. For this reason temperature-humidity relations would be different, also the weather was somewhat warmer at the time of the first experiment. In any case, the significance of the results of both experiments is the same. The data show increased plant response to increased concentration of chemical. The 2,4-D or some reaction product moved both down and up in the plant tissues and, as far as could be ascertained visually, it remained confined to the extreme upper regions of the plants with no effect on lower stems and leaves or on roots. Plants were examined periodically for nine weeks until the end of the season.

*Effect of Treatments on Seed Production*

Again as in Expt. I, there was practically complete sterility of flower heads of treated and untreated plants. The bagging practice seems to have been responsible for the sterility but since the main observations on direction and localization of movement of chemical were made before the inflorescences were covered they were free from the influence of this complicating factor.

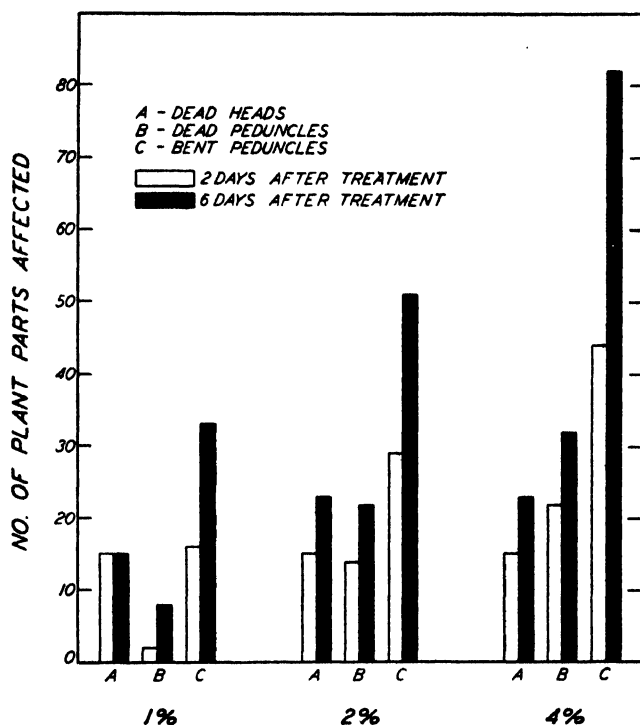


FIG. 1. Total number of affected parts on 15 sow thistle plants two and six days after treatment of one flower head per plant with 0.1 ml. of solution of morpholine salt of 2,4-D (1%, 2%, and 4% solutions as 2,4-D acid equivalent).

## 2. INJECTION OF CUT STEMS

*Experiment III**Materials and Methods*

Sow thistle plants of the same stock and plot location as those treated in Expt. II were used in a concurrent experiment to observe the effects, on remaining plant parts, of 2,4-D injected into the stump after cutting off the upper portions of the plant stem. The main stem was cut off about 8 to 10 in. above ground level and whenever branching pattern permitted, two lateral branches arising near the base of the stem were left complete with their developing flowers.

Aliquots of the 1%, 2%, and 4% solutions of morpholine salt used in the foregoing experiment were delivered by means of a graduated 1 ml.

hypodermic syringe into the central cavities of the respective stem stumps. A single 0.4 ml. aliquot was used for each plant, thus in this experiment stem injections of 0.4 ml. of 1% solution were equivalent in 2,4-D acid content to the flower injections of 0.1 ml. of 4% solution in Expt. II. Where pith obstructed the stem cavity it was first pierced by a wire probe. Each treatment, including untreated plants and controls treated with distilled water was replicated 15 times. Stems were sealed with scotch tape after treatment.

### Results

Two days after treatment, browning of the exterior of the stem near place of application of 2,4-D was a common symptom of injury among the plants treated with the 2% and 4% concentrations. In general, plants were affected in proportion to concentration of 2,4-D applied.

There was naturally some variability among plants even though they were of the same age. Smaller somewhat more succulent lateral branches were affected most rapidly. They curled significantly indicating movement of reactant down the treated stump and up the branches to the developing flowers.

Curling of leaves and branches was followed by loss of chlorophyll and appearance of mottled red and yellowish coloration of leaves. This changed to brown, marking the death of affected aerial parts (See Figs. 2-8).

Some of the observations recorded over a period of nine weeks are summarized in Table II.

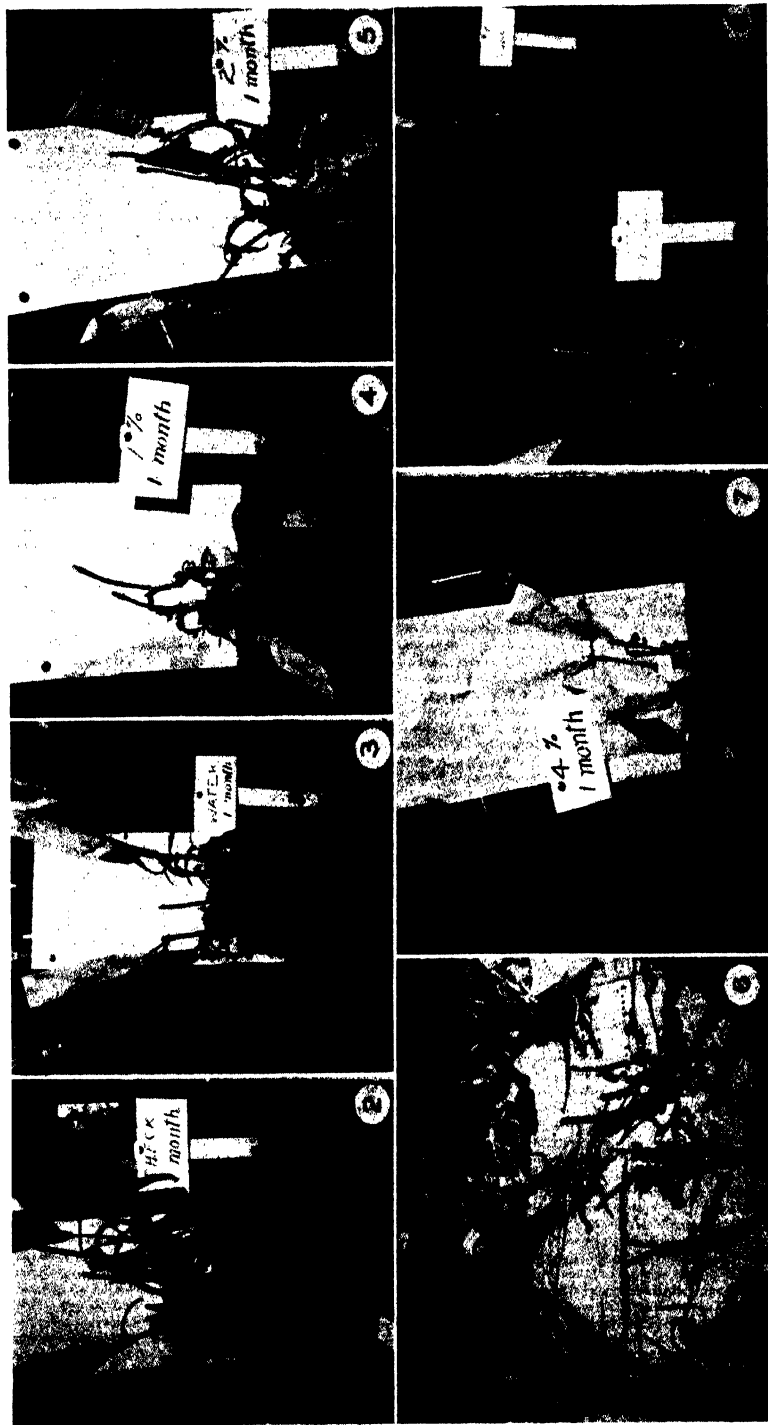
TABLE II

NUMBER OF SOW THISTLE PLANTS PER GROUP WITH ALL AERIAL PARTS DEAD FOLLOWING STEM INJECTION AT FLOWERING TIME WITH 2,4-D SOLUTION (15 PLANTS PER GROUP)

Treatment	Weeks after treatment			
	4	6	7	9
2,4-D solutions				
1%	6	6	6	12
2%	6	10	12	14
4%	9	11	13	14
Water	0	0	0	10 (dying)
Check	0	0	0	8 (dying)

Nine weeks after treatment (Aug. 6-Oct. 6) the untreated and water-treated controls were beginning to die from natural causes. As the data indicate, however, they were still in better condition than were the treated plants. In addition, a considerable amount of new growth had sprung up from pruned branches of the control plants. No such growth appeared on treated plants (See Pl. I).

As also noted in Expts. I and II, bagged flower heads left to mature on lateral branches of treated and control plants produced no seed.



*Appearance of severed sow thistle plants after stem injection.*

FIG. 2. Check, one month from time of treatment. FIG. 3. Check: distilled water, one month from time of treatment. FIG. 4. 1% 2,4-D solution, one month from time of treatment. FIG. 5. 2% 2,4-D solution, one month from time of treatment. FIG. 6. Check plants (above) and comparable 2,4-D treated plants (below) six weeks after treatment. FIG. 7. 4% 2,4-D solution, one month from time of treatment. FIG. 8. Check plant (foreground) and plant treated with 4% 2,4-D solution (background) nine weeks from time of treatment.



Nine weeks after treatment part of the root system of five plants from each group was removed by digging to a depth of one foot in a cylinder of soil 33 in. in diameter having the plant stem as its central point. Care was taken to avoid, in so far as possible, breakage of roots from the parent plant, and to include all portions disconnected as a result of rotting of treated material. Although the technique was subject to error, the differences recorded in Table III are in line with visual observations of lessened vigor and substance of roots from treated plants (See Pl. I).

TABLE III

FRESH WEIGHT AND APPARENT CONDITION OF SOW THISTLE ROOTS IN TOP FOOT OF SOIL WITHIN A RADIUS OF 16½ IN. FROM PLANT STEM NINE WEEKS AFTER INJECTION OF CUT STEMS WITH 2,4-D SOLUTIONS

Treatment	Root wt., gm.				
	Check	Water	1% 2,4-D	2% 2,4-D	4% 2,4-D
Plants 1	11	50	24	17	0
2	33	22	19	10	8
3	34	18	31	6	9
4	26	18	8	5	8
5	37	37	31	4	3
Total	141	145	113	42	28
Mean	28	29	23	8	6
Estimated % dead roots	0	0	25	40	70
Estimated % extensively deteriorated roots	0	0	45	35	25

In addition to the dead material, several of the roots were severely damaged throughout their length in the volume of soil examined. As one would expect, damage decreased with distance from the plant. Affected roots were very thin, spongy, brown, and knobby. About one quarter of the extensively deteriorated roots, however, still possessed slightly enlarged resting buds but it seems likely that these were of greatly reduced vitality. Further observation and experiments are necessary to settle this point.

### Discussion

Results, following injection of flower heads of perennial sow thistle plants with 2,4-D, show that there was a downward movement of herbicide through peduncles supporting the treated heads. It seems likely that opposition to such movement would be decreased as development of flower heads became arrested. On the other hand, the descent of chemical was apparently completely stopped whenever avenues for its upward movement in neighboring peduncles were reached. It seems that transport of reacting chemical was then governed by movement of sap to the young rapidly developing parts of the plant. This is clearly shown by the numbers of dead flowers and of dead



or twisted peduncles. Apparently movement of herbicide in the peduncles was not confined to any particular tissue region. Highest concentrations of chemical produced most response.

As far as could be ascertained visually, there was complete lack of response below the uppermost region of head-injected plants. Two months after treatment the appearance of the remainder of the aerial portion and of the entire roots was still indistinguishable from the controls.

The evidence for multidirectional transport is supported by the results of injection of sow thistle stem stumps. Movement down the stem resulted in death of a high proportion of roots. In addition, some of the chemical moved upwards in intact lateral branches, entered their leaves, produced characteristic symptoms of growth disturbance within 24 hr. from treatment, and caused damage as indicated in Tables II and III and Pl. I.

Because of differences in technique the experiments on flower injection are not strictly comparable with the test in which severed plants were used. Nevertheless, some comparisons appear to be admissible and instructive. In quantity of 2,4-D applied, the acid content of the amount of 4% solution used for flower treatment of one group of plants was equivalent to the acid content of the greater quantity of 1% solution used in stalk treatment of another group of plants. It has already been shown that the response to the latter treatment was appreciably greater than that resulting from injection of flower heads. In no case did death of a portion of a plant, anywhere nearly equivalent in mass to that killed by stalk treatment, occur after flower treatment. Admittedly there was better opportunity for immediate dispersal of 2,4-D inside the cut stems but it also seems logical to assume that as a result of cutting the stems the upward movement of liquids within the plant was reduced, thus permitting 2,4-D solution to move more extensively downwards. It has already been noted that upward movement occurred in intact lateral branches, leaves, and flower peduncles. According to the theories discussed in the introduction we would not expect appreciable upward movement through phloem of leaves functioning under favorable condition, as these were. It seems logical to assume, therefore, that ascent of herbicide into leaves occurred through their xylem.

Now if the behavior of entire and of severed flowering sow thistle plants following spraying or dusting of their leaves and stems with herbicide is found to be similar to that noted in the experiments described above, then an immediate practical application of the findings would suggest itself. Mowing of infested uncropped land at a time when sow thistle root reserves are lowest (1) could be done at a height to leave adequate leaf surface for spraying. Mowing would also help to control certain intermingled annual weeds such as red root pigweed, which is moderately resistant to 2,4-D.

We are not aware of any published comparative results of spraying at flowering stage of intact and mowed sow thistle plants. Trials of this nature are anticipated.

### Summary and Conclusions

Injection of single new flower heads of perennial sow thistle plants with herbicidal concentrations of 2,4-D resulted in localized response to treatment. The stimulus moved down the peduncles of treated flower heads and up several neighbouring peduncles indicating that translocation of 2,4-D is associated with flow of liquid to active centers of development or of transpiration. Apparently movement of herbicide in peduncles was not confined to any particular tissue region. Severity of response varied directly with concentration of 2,4-D used.

Injection of severed stems of perennial sow thistle plants with 2,4-D resulted in extensive kill of intact lateral branches and leaves and of root growth. Multidirectional movement of selective herbicide is thus shown to occur. Apparently ascent of herbicide into leaves occurred through their xylem. It is suggested that abscission of stems and flowers results in a reduction of the normal upward movement of liquids thus permitting more extensive downward movement of herbicide, perhaps associated with translocation of nutrients to storage areas.

If the above results are applicable under practical conditions of application of selective herbicide, it would be advisable, prior to chemical treatment of flowering sow thistle plants growing on uncropped land, to mow them at height of several inches above ground.

### Acknowledgment

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# LETHAL EFFECT OF ABSORBED RADIOISOTOPES ON PLANTS<sup>1</sup>

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## Abstract

The lethal dose of  $P^{32}$  and  $Sr^{90}$  for wheat, barley, and sunflower seeds is approximately 1.4 microcuries per seed (0.05 rd.). Radioautographs indicate that at five weeks' growth the distribution of phosphorus in a wheat plant is fairly uniform whereas that of strontium is very uneven, practically all the strontium being concentrated in the first two leaves.

## Introduction

It is now well known that radiations can produce marked physiological effects in living cells. Consequently, it is important when conducting tracer experiments with biological material, to keep well below the biological threshold, for otherwise the conclusions drawn may be invalidated by occurrences due to the radiation itself. A good deal is already known concerning the action of X rays,  $\gamma$  rays, and  $\beta$  particles on living cells (4) but very little has been published concerning the action on plants of beta rays from radioactive tracers.

The present experiments were designed to measure the lethal dose of radio-phosphorus,  $P^{32}$ , and radiostrontium,  $Sr^{90}$ , for germinating seeds of wheat species, barley, and sunflower. The experiments grew out of tracer studies in which  $P^{32}$  was used to measure the uptake of phosphate fertilizer by wheat plants (8).

## Experimental

### METHOD

#### *Method of Counting*

The activities of solutions were determined by evaporating down an aliquot on a platinum dish that was then slid under the window of an end-on Geiger Müller chamber, having a thin window of mica, and counted. The chamber was connected to a scale of 128 scaling circuit and any sample was counted for a sufficient length of time to give approximately 10,000 counts (standard deviation is then about 1%). By choosing a suitable aliquot the rate of counting was kept below 2000 counts per minute and the correction for resolving time of the tube ( $2 \times 10^{-4}$  sec.) was then less than 1% (10).

Plant materials were wet ashed before counting and, where necessary, corrections were made for self-absorption by the active material (8). The

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natural rate of the counting tube (so-called 'background') was allowed for by subtracting it from the total counting rate for any sample. For  $P^{32}$ , half life 14.3 days, the radioactive decay was allowed for by taking an aliquot of the original stock solution, evaporating it to dryness, and counting it immediately before and after counting the unknown material. The aliquot of the original thus serves as a standard and eliminates the necessity of correcting for the decay of the  $P^{32}$  or for small fluctuations in the counter.

For  $Sr^{90}$  (half life 25 years (7)), counter fluctuations were allowed for by comparing the unknown with a uranium oxide standard.

#### *Absolute Number of Disintegrations*

In determining the lethal dose, absolute tracer activity is required. While methods are available for the measurement of absolute disintegration rates (3) a simpler procedure is to use a  $\beta$ -ray standard, preferably identical in energy distribution with those being measured. In the present experiments, the factor converting recorded counts to absolute number of disintegrations was determined using a radium D + E standard obtained from the Bureau of Standards, Washington. The counting efficiency of the counter for the radium D + E standard was first determined and then the disintegration rate of the  $P^{32}$  standard solution measured by evaporating an aliquot on the surface of a 1 in. silver disk similar to the one on which the standard radium D + E was mounted. Another aliquot was prepared under the conditions under which routine samples were measured, namely in a platinum dish. The ratio of the disintegration rate to the observed counting rate gave the geometry factor for  $P^{32}$  under routine counting conditions. The geometry factor for  $P^{32}$  for these experiments was 3.9. The corresponding geometry factor for  $Sr^{90}$  (+ Y daughter) was also 3.9.

In accordance with a recent suggestion of Condon and Curtiss (2) the activity has been expressed in rutherfords (rd.), one rutherford being defined as  $10^6$  disintegrations per second.

#### *Radiophosphorus, $P^{32}$*

The  $P^{32}$  was in the form of disodium hydrogen phosphate, about 4 mgm. disodium hydrogen phosphate per mc. of activity of  $P^{32}$ . This was dissolved in water to give a solution of activity 0.65 rd. per ml. Solutions of lower activities were made by dilution of the stock solution.

#### *Radiostrontium, $Sr^{90}$*

The stock solution of  $Sr^{90}$  was in the form of a 0.3% solution of strontium chloride pH = 4.7. The  $Sr^{90}$  was in equilibrium with its Y daughter and the beta activity (11.7 rd. per ml.) is thus due equally to  $Sr^{90}$  (0.6 mev.) and  $Y^{90}$  (2.6 mev.). Solutions of lower activities were made by dilution of the stock solution.

## Results

### *Lethal Concentration of Disodium Hydrogen Phosphate for Triticum vulgare Vill. var. Thatcher*

Seeds of Thatcher wheat were germinated in water for 48 hr. and at the end of this time they were placed in test tubes containing a known amount of  $P^{32}$ , obtained by evaporating to dryness, in the bottom of the test tube, aliquots of a standard solution of active disodium hydrogen phosphate. Into this was delivered 0.1 cc. of Knop's nutrient (5) that had been made up lacking the usual dipotassium hydrogen phosphate. The following test tubes were set up:

Series No. 1; eight test tubes, zero radioactivity, controls.

Series No. 2; eight test tubes, 0.0065 rd. radioactivity.

Series No. 3; eight test tubes, 0.065 rd. radioactivity.

The seedlings were left until the test tubes were perfectly dry and were then placed on blotting paper shelves in clean test tubes containing 5 cc. Knop's nutrient. The seeds dried at various times but the transfer was complete by the fourth day. Most members of series No. 2 were transferred on the second and third days while the individuals from series No. 3 were transferred entirely on the fourth day.

The test tubes from which the plants had been removed were rinsed with water, then with acid, and the rinsings evaporated and counted under the Geiger counter. The average radioactivity remaining in series No. 2 was 2% but in series No. 3 it was 20%. Possibly, in this case, the fact that the plants were growing more slowly gave more of the liquid an opportunity to evaporate and remain on the walls of the test tube.

Root lengths were measured twice a day. Fig. 1 (inset) shows the variation in growth of the three groups. Average figures from each group of eight were used. At the end of the second day the roots were too coiled for further measurement and shoot lengths were measured. Fig. 1 shows the growth variation of the shoots from the 2nd to the 12th day. The individuals of series No. 3, with the exception of one, did not grow at all after being introduced into the solution but remained exactly the same length as they had been following germination in water. The one individual that grew achieved a length of 0.5 cm. and then remained stationary, finally dying. The results of this experiment suggest that a disintegration rate of 0.05 rd. ( $0.065 \times 0.8$ ) of beta particles from  $P^{32}$  (approximately 1.4 microcuries) is lethal to a Thatcher seedling. From the size of the wheat seed we can estimate that about 50% of the beta particles from the  $P^{32}$  will be absorbed by the seed (see data for actual measurements on  $Sr^{90}$  later). The average energy of the beta particles is 0.7 mev. and thus the energy dosage per seed is  $\frac{1.4}{2} \times 3550$  ergs per day = 2485 ergs per day =  $\frac{2485}{83}$  r.e.p.\* per day = 30 r.e.p. per day. For a seed

\* r.e.p. = roentgen-equivalents-physical ( $\delta$ ).

of 0.3 gm. weight this corresponds to 100 r.e.p. per gm. of tissue per day (3, p. 259).

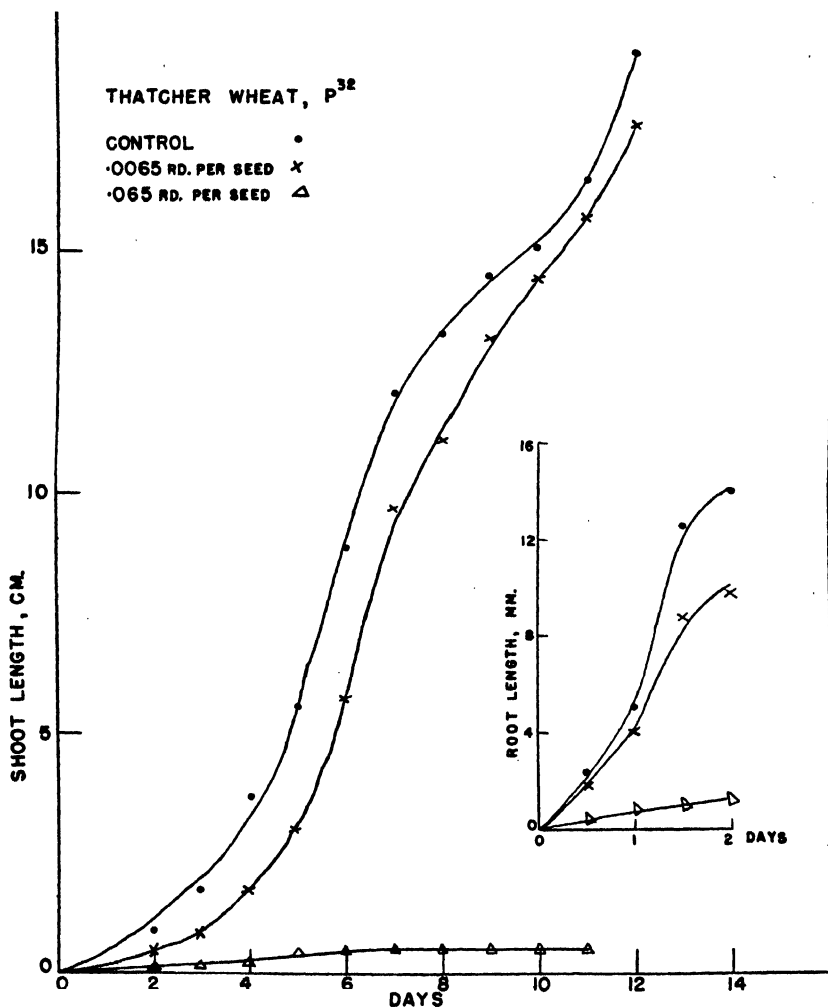


FIG. 1. Plot of average shoot length versus period of growth for Thatcher wheat containing radioactive phosphorus,  $P^{32}$ . Inset: root length in mm. versus time in days for initial stages of growth of Thatcher seeds containing  $P^{32}$ .

#### Effect of $P^{32}$ on Thatcher, Pelissier, and Einkorn Wheats and Hannchen Barley

Additional information is given in an experiment set up mainly to provide information on the cytological and genetic effects of  $P^{32}$  on the following plants (1):

*Triticum vulgare* Vill. var. Thatcher ( $n = 21$ )

*Triticum durum* Desf. var. Pelissier ( $n = 14$ )

*Triticum monococcum* L. (Einkorn,  $n = 7$ )

*Hordeum distichon* L. var. Hannchen ( $n = 7$ )

On July 8, 1947, test tubes were set up to receive the seeds as follows:

- Einkorn, 24 test tubes, radioactivity 0.0065 rd.
- Einkorn, 24 test tubes, radioactivity 0.00065 rd.
- Einkorn, six test tubes, radioactivity zero (control).
- Pelissier, 24 test tubes, radioactivity 0.0065 rd.
- Pelissier, 24 test tubes, radioactivity 0.00065 rd.
- Pelissier, six test tubes, radioactivity zero (control).
- Thatcher, 24 test tubes, radioactivity 0.0065 rd.
- Thatcher, 24 test tubes, radioactivity 0.00065 rd.
- Thatcher, six test tubes, radioactivity zero (control).
- Barley, 24 test tubes, radioactivity 0.0065 rd.
- Barley, 24 test tubes, radioactivity 0.00065 rd.
- Barley, six test tubes, radioactivity zero (control).

On July 10, seeds that had been germinated for 48 hr. were placed in the test tubes as listed above and appropriate amounts of radioactive solution added. The controls were given an equal quantity of tap water. By July 12, the radioactive solution had been absorbed by most individuals and Knop's nutrient was added. The Knop's was made up according to the usual formula except that the dipotassium hydrogen phosphate was omitted. The controls were given regular Knop's nutrient. Growth curves (average) for these plants are shown in Figs. 2 to 5. Owing to extreme heat these curves may not represent normal behavior although, at maturity, they were close enough to normal

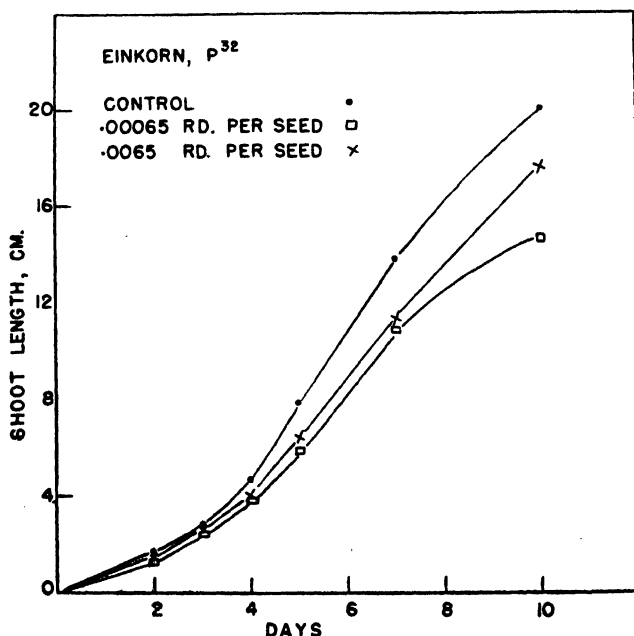


FIG. 2. Plot of average shoot length versus period of growth for Einkorn wheat containing radioactive phosphorus,  $P^{32}$ .



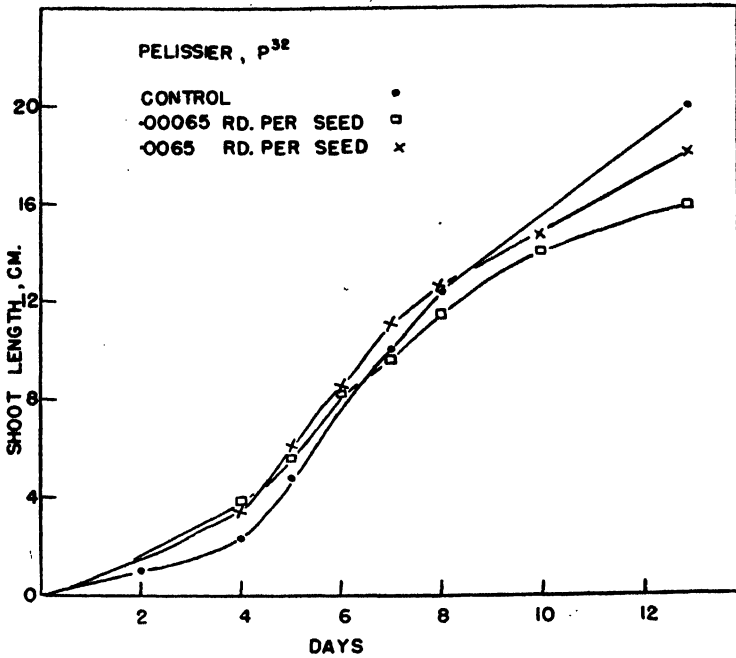


FIG. 3. Plot of average shoot length versus period of growth for *Pelissier* wheat containing radioactive phosphorus,  $P^{32}$ .

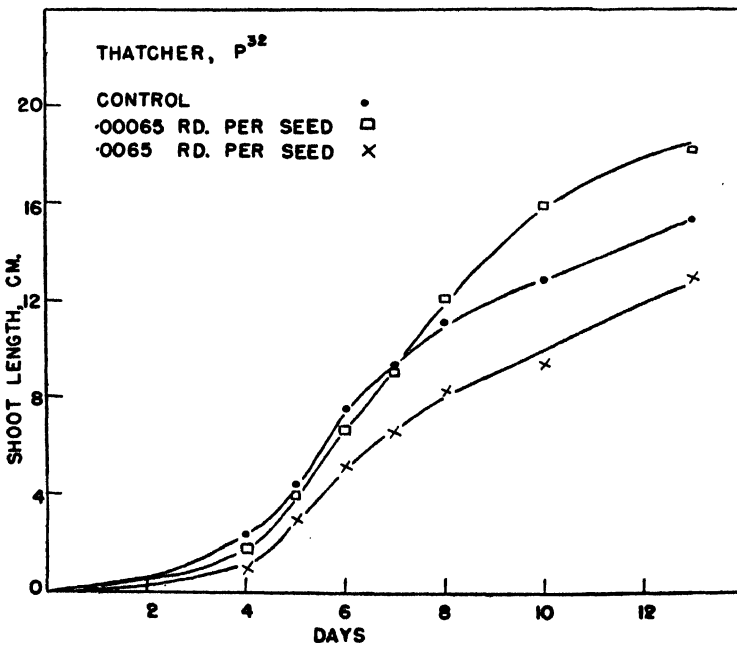


FIG. 4. Plot of average shoot length versus period of growth for *Thatcher* wheat containing radioactive phosphorus,  $P^{32}$ .

appearance that it may be assumed that the heat had no permanent effect upon the growth of the plants. The standard deviation for any given set of shoot length measurements was about 20%. It appears from these experiments that, for  $P^{32}$ , a beta activity of 0.0065 rd. is below the lethal limit for the three varieties of wheat and the one variety of barley. This is in agreement with the previous wheat experiment.

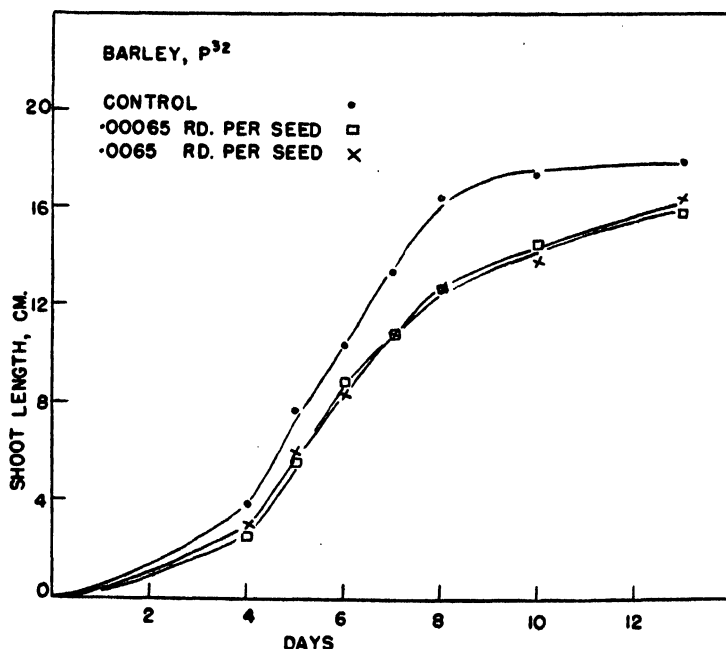


FIG. 5. Plot of average shoot length versus period of growth for Hannchen barley containing radioactive phosphorus,  $P^{32}$ .

#### Lethality Experiments for $Sr^{90}$ (+ Y Daughter) on Wheat, Barley, and Sunflower

Five types of seed were investigated: Einkorn, Pelissier, and Thatcher wheats, Regal barley, and sunflower (an inbred variety of *Helianthus annuus* L.,  $n = 17$ ). Germination tests were run on the five plants. One hundred seeds were placed on soaked Kleenex and germination read on the first and second days. Germination had taken place in over 90% of the seeds of each variety, with the exception of sunflower, at the end of 48 hr. The sunflower showed 74% germination at this time and 100% in 72 hr.

One hundred and fifty test tubes were set up in racks. The racks were darkened with black paper walls to keep some of the light from the roots. Each of the five types of seeds were set up as follows:

- Six seeds in 0.1 ml. solution each, radioactivity 0.65 rd.
- Six seeds in 0.1 ml. solution each, radioactivity 0.065 rd.
- Six seeds in 0.1 ml. solution each, radioactivity 0.0065 rd.
- Six seeds in 0.1 ml. solution each 0.33% inactive strontium chloride.
- Six seeds in Knop's nutrient.

The most highly radioactive solution was 0.33% with respect to strontium chloride, the other two, 0.033% and 0.0033% respectively. The seeds in Knop's nutrient were started on blotting paper shelves but all the rest were placed in the bottom of the test tubes and 0.1 ml. of solution delivered on top of them. When the seeds had absorbed all the moisture available they were carefully removed with forceps and placed on blotting paper shelves and 5 ml. Knop's nutrient added to each test tube.

Counts of the number germinated in each group were made every day. Measurements of shoot length were also made and averages recorded. Fig. 6, for Einkorn, is typical of the curves obtained. On the 13th day after the

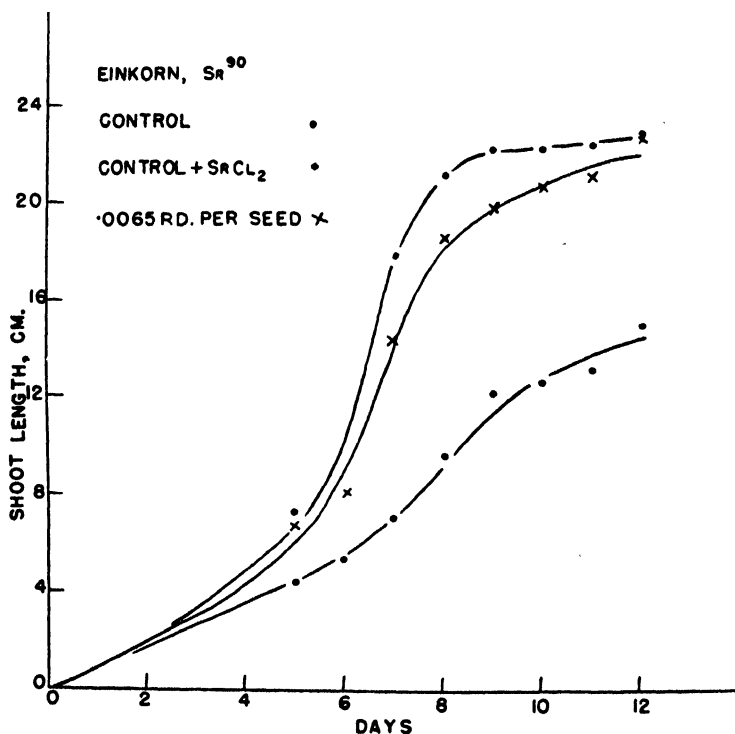


FIG. 6. Plot of average shoot length versus period of growth for Einkorn wheat containing radioactive strontium,  $Sr^{90}$  (plus  $Y^{90}$  daughter).

seeds were started the plants were transferred to crocks and grown in the greenhouse. The residual  $P^{32}$  in the solution was measured and indicated that the seeds had absorbed approximately 80% of the radioactivity.

The seeds given 0.65 rd.  $P^{32}$  did not grow at all, those that were started at 0.065 rd. germinated very poorly and all died although one hardy Einkorn plant grew a stem, but no roots, for a week before perishing.

The figures therefore show growth curves for 0.0065 rd. activity and the two controls. The sunflower group was an exception, appearing to withstand the higher activities better both in germination and growth. Since

it was thought that this difference might have been due in part to the thick husk of the seed, further experiments were done with the outer husk removed (see later).

These experiments indicate that an activity of 0.065 rd. of  $\text{Sr}^{90}$  (+  $\text{Y}^{90}$ ) is lethal for the above named varieties of wheat and barley whereas 0.65 rd. is lethal for sunflower.

After the experiment was in progress the pH of the various solutions was measured. The pH of the final solutions ranged from 6.5 to 8.0 but the pH of the most active initial solution was 3.8. Since the lethal effect of the most active solution might have been due in part to the pH, a further set was run in which care was taken to have the pH of even the most active solutions somewhat higher (4.7).

*Further Lethality Experiments for  $\text{Sr}^{90}$  (+  $\text{Y}^{90}$ ) on Wheat, Barley, and Sunflower*

Additional experiments were done with Thatcher wheat, barley, and sunflower. One set of each of these was allowed to germinate and grow for 24 hr. prior to being subjected to the radioactivity. Another set of the same species was subjected to the radioactivity immediately so that germination had to take place in the radioactive solution. These sets were run in series of five seeds each.

Series 1. Five seeds of Thatcher, 0.1 ml. solution each:  
radioactivity 0.65 rd.

Series 2. Five seeds of Thatcher, 0.1 ml. solution each:  
radioactivity 0.065 rd.

Series 3. Five seeds of Thatcher, 0.1 ml. solution each:  
radioactivity 0.0065 rd.

Series 4. Five seeds of Thatcher, 0.1 ml. solution each:  
radioactivity 0.00065 rd.

Series 5. Five seeds of Thatcher, 0.1 ml. solution each:  
inactive  $\text{Sr}^{++}$ .

This was also done with barley and sunflower, using pregerminated and direct treatments. In addition, a peeled set of sunflower seeds was subjected to a similar treatment.

*Procedure*

In the pregerminated series, seeds of the three species were allowed to germinate for 24 hr. and after measurement of the size of the root tip they were inserted into test tubes and 0.1 ml. of the various activities of strontium chloride solution added. In the direct series, 0.1 ml. of solution was added directly to the ungerminated seed in a test tube. Controls were treated similarly, 3 mgm. inactive strontium chloride per ml. being used, this being slightly in excess of the concentration of radioactive strontium chloride. After about 60 hr., modified Knop's solution (in which phosphates and sulphates were eliminated to prevent precipitation of the strontium ion) was

added. The growth was measured every 24 hr. and the average growth calculated and recorded. Fig. 7, for Thatcher, direct treatment, is typical of the curves obtained. The average growth after 310 hr. is tabulated in Table I.

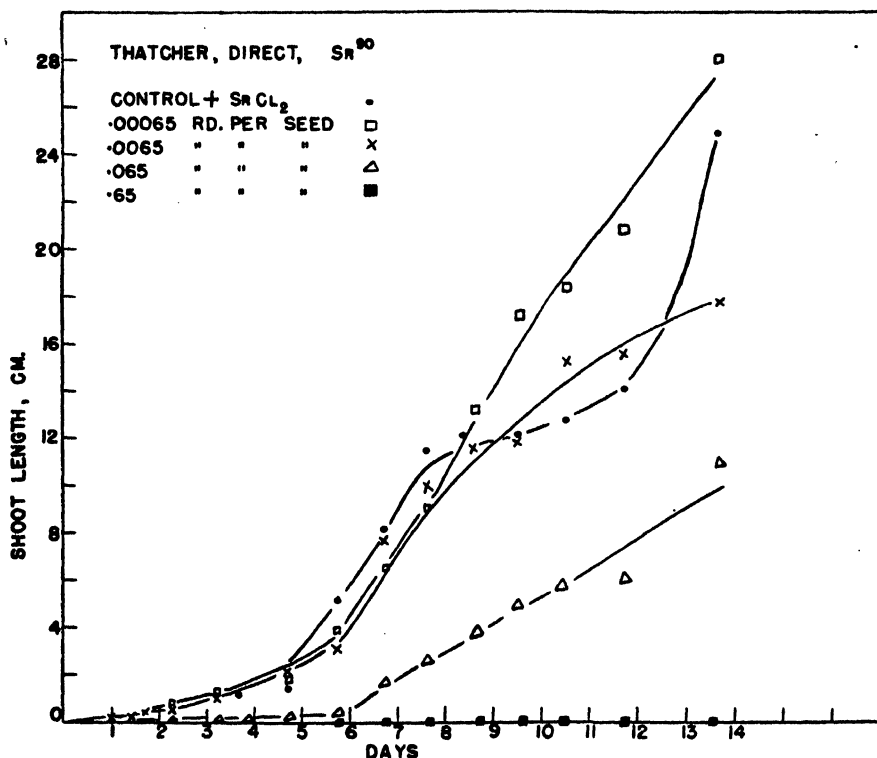


FIG. 7. Plot of average shoot length versus period of growth for Thatcher wheat containing radioactive strontium,  $Sr^{90}$  (plus  $Y^{90}$  daughter). Active solution applied directly to the ungerminated seed.

TABLE I

AVERAGE GROWTH AFTER 310 HR. (CM. FROM TIP OF MAIN ROOT TO TIP OF TOP LEAF)

Radioactivity (rd.)		0.65	0.065	0.00065	0.00065	Control
Thatcher	P.G.*	0.12	11.6	19.6	21.2	22.0
	D.**	0.0	7.0	18.0	24.8	27.5
Barley	P.G.	0.12	12.0	18.4	21.0	21.5
	D.	0.0	12.2	17.9	21.0	22.0
Sunflower (unpeeled)	P.G.	0.1	6.8	13.1	11.2	14.0
	D.	0.0	1.5	13.6	8.8	15.0
Sunflower (peeled)	P.G.	0.1	9.0	10.4	12.2	13.5
	D.	0.0	2.5	11.2	11.5	10.0

\* P.G., pregerminated.

\*\* D., direct.

In these experiments, it appears that 0.65 rd. is completely lethal in both direct and pregerminated experiments while 0.065 rd. is strongly inhibitory. Inhibition appears to be somewhat greater in the direct than in the pregermination experiments. The amount of  $\text{Sr}^{90}$  absorbed in these experiments was not measured so that the above figures give only an approximate lethal limit. The plants appeared to have a somewhat greater resistance to activity than in the first strontium lethality experiments, possibly owing to the more favorable pH in this set of experiments, but possibly owing also to slightly different growing conditions. At all events, we can say that the lethal limit is not very different from that for  $\text{P}^{32}$  and is about 0.1 rd. per seed. Cytological effects were noted and are reported elsewhere.

The Thatcher, barley, and sunflower seeds weighed 0.29, 0.29, and 0.66 gm. respectively. In order to estimate the fraction of beta particles absorbed by the seed the decrease in activity of a small evaporated drop of  $\text{Sr}^{90}$  (+ Y) solution, on being covered by a split seed, was measured. About 40% of the beta particles were absorbed by Thatcher and barley and 55% by sunflower. Before making any exact calculations, the distribution of activity in the seed at various times is required and it is proposed to measure this in future experiments. One would also need to estimate the absorption, by the seed, of  $\beta$ -particles from the solution.

#### *Toxicity of Inactive Strontium Solutions*

One hundred seeds of Thatcher wheat were germinated in each of the following solutions:

- |   |          |
|---|----------|
| 1. Strontium chloride                         | 0.01%    |
| 2.            "                               | 0.08%    |
| 3.            "                               | 0.5%     |
| 4.            "                               | 1.0%     |
| 5. Strontium nitrate                          | 0.01%    |
| 6.            "                               | 0.08%    |
| 7.            "                               | 0.5%     |
| 8.            "                               | 1.0%     |
| 9. Tap water                                  |          |
| 10. Modified Knop's nutrient made as follows: |          |
| Strontium nitrate                             | 0.08 gm. |
| Potassium nitrate                             | 0.04 gm. |
| Magnesium nitrate                             | 0.02 gm. |
| Ferric nitrate                                | Trace    |
| Water   | 100 cc.  |

The concentrations were chosen to straddle the concentration of 0.33%, previously used with radioactive strontium chloride, so as to determine whether or not the presence of the salt contributed to the lethal effects observed. The concentration of 0.08% was chosen because it is the percentage used in the modified Knop's solution. Germination results were as follows:

## Germination, %

Salt		After 24 hr.	After 48 hr.	After 72 hr.
Strontium chloride	0.01%	50	94	98
	0.08%	74	98	98
	0.5%	78	97	97
	1%	21		
Strontium nitrate	0.01%	79	75	87
	0.08%	54	97	97
	0.5%	22	97	99
	1%	44	90	96
Tap water		30	86	93
Modified Knop's		34	92	97
			88	97

From these results it can be concluded that as far as germination is concerned, the only salt showing any appreciable effect is strontium chloride, 1%. Here there seems to be a definite, though small, inhibition of germination. It appears fairly certain that the 0.33% concentration used in the work described would have no retarding effect on germination.

Trelease and Trelease report in their investigation of magnesium injury of wheat (9) that the characteristic deformities of this injury do not manifest themselves until the appearance of the third leaf. Accordingly it was decided to raise two germinated seeds from each group in test tubes.

The seedlings were placed on blotting paper shelves and 10 ml. of solution, of the same concentration as that in which they were germinated, was added so that their growth habit in this solution could be watched.

Up until the 10th day of growth no adverse effects were noted on Thatcher seedlings grown in approximately 0.3% solutions of stable strontium nitrate and stable strontium chloride. Seedlings grown in 1% solutions of these compounds showed retarded growth and all died between the 24th and 32nd days. On the 32nd day the condition of the individuals was as follows (figures are average of two individuals):

Solution	Conc., %	Height of plant, cm.	Appearance
Strontium chloride	1	—	Dead
"	0.5	13	First leaf yellow
"	0.08	15	First leaf brown
"	0.01	19	First leaf yellow
Strontium nitrate	1	—	Dead
"	0.5	14	First leaf yellow
"	0.08	15	First leaf dead
"	0.01	18	First leaf brown
Tap water		24	Healthy
Modified Knop's (strontium replacing calcium)		28	Healthy

The number of individuals used in this experiment does not permit definite conclusions with regard to the ultimate toxicity of strontium compounds upon Thatcher wheat, but the results indicate that retardation of growth in the early seedling increases with increasing concentration of the strontium salt used. Further, a concentration of strontium nitrate of 0.08% apparently exerts no effect when used in a nutrient solution containing magnesium salt.

The injury sustained by the plants, apart from stunting, was uniform. The first leaf became yellowed, then withered and died. The second and third leaves were healthy. This suggests that the plant removed the strontium from the solution and that the building up of the concentration in the first leaf was lethal to this leaf.

#### *Distribution of Radioactive Elements in Plants*

An attempt was made to compare qualitatively the distribution of  $\text{Sr}^{90}$  and  $\text{P}^{32}$  ions in Thatcher wheat.

The seedlings and plants used in this study were obtained from previous experiments on the lethal effects of the two radioactive isotopes mentioned. The seeds were germinated in water for 48 hr., removed to test tubes, and treated with radioactive solutions with activities of the order of  $4 \times 10^6$  disintegrations per minute.

Radioautographs were obtained by fixing the plant to a glass plate with cellophane tape and then placing it on a Process film, the plant being adjacent to the emulsion side of the film. The film was exposed for 48 hr. and then developed with Kodak D72 developer.

The radioautograph of a 12-day-old wheat seedling grown in  $\text{P}^{32}$  solution showed that the phosphorus was distributed fairly uniformly throughout the whole plant and that the activity was particularly high in the seed. The radioautograph of a 35-day-old plant in  $\text{P}^{32}$  showed a general distribution of activity, what little concentration there was occurring at the leaf tip. There appeared to be no difference between young and old leaves. The radioautograph of a 40-day-old plant, which had been treated with radioactive strontium chloride in its early stages of growth and then grown in soil in a pot, showed quite a different result. The activity was all concentrated in the first two leaves of the plant, the concentration in the first leaf greatly exceeding that in the second. It thus appears that the plant gets rid of the strontium chloride, not a normal plant nutrient, in the first two leaves.

#### **Acknowledgments**

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## A SUBSTANCE IN THE INTERCELLULAR SPACES OF WHITE MUSTARD ROOTS THAT REDUCES ALKALINE SILVER<sup>1</sup>

BY R. G. H. CORMACK<sup>2</sup>

### Abstract

White mustard roots when treated with a slightly alkaline solution of silver nitrate (Tollen's reagent) show, on sectioning the meristem, the accumulation of small black stained particles in the intercellular spaces and intervening cell walls. The production of these particles in the intercellular spaces of treated roots and their complete absence in those of untreated roots indicates the presence of a substance capable of reducing alkaline silver. The presence of this substance strengthens the viewpoint that in the meristem radiating rows of intercellular spaces in white mustard roots provide for the more rapid movement of soluble materials from differentiating vascular strands to the outside. Thus cell position as related to a more accessible food supply is an important adjunct to the general problem of cell development and cell behavior.

### Introduction

In order to synthesize new protoplasm, meristematic cells require a continuous supply of food. How this is accomplished in the apical meristem of the root, where the dividing cells are situated some distance beyond the vascular system, is a problem that has provoked considerable interest. Although it is now well established (5, 6, 7, 15) that the sieve tubes differentiate well down into the meristematic tip it is still a matter of conjecture how the soluble materials that they carry reach the actively dividing cells. In a long series of publications (8, 9, 10, 14), Priestley and his students conclude that the intercellular spaces and cell walls are the natural channels of food transport from the ends of differentiating vascular strands to the dividing cells. In accordance with this view it follows that the meristematic cells nearest the source of supply will be better nourished than those situated near the surface.

Certain observations obtained in a study of developing epidermal cells in white mustard roots lend considerable support to this view of food transport as visualized by Priestley and his school of workers. In the root of this plant (3) the epidermis is differentiated at an early stage into alternating rows of long and short cells. The long cells vacuolate early and do not ordinarily produce root hairs, while the short cells vacuolate later and to a lesser extent and produce hairs normally. In a later study (4) these differences between short and long cells are explained on a basis of differences in cell position as related to a more accessible food supply. For instance, as seen in transverse view each short cell is situated in direct line with a row of intercellular spaces within the cortex, while the long cells are not associated with intercellular spaces at any time in their development.

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Although the existence of a 'nutritive sap' is highly probable, no one, as far as the present writer is aware, has demonstrated the actual presence of soluble materials in the intercellular spaces. Because of the delicate nature of the root meristem and the extremely small size of the intercellular spaces it is obvious that the problem is not an easy one. The solution of this problem would be infinitely easier if it were possible to test directly for solutes in the spaces of living tissue, but this necessitates cutting, which means the escape of liquids from the spaces and their rapid spread over the cut surface. Another method is to produce a chemical reaction within the spaces leaving the root intact. This method has its difficulties too. For instance, many reagents that give a definite reaction *in vitro* are not suitable when applied to plant tissue, either because of their destructive action on the protoplasm and cell walls or because the reaction requires strong heating, which has the same effect. Moreover because of the small size of the spaces there may not be a sufficient amount of the substance for which the test is being made to produce a positive reaction.

Since the existence of a 'nutritive sap' in these spaces is speculative and since nothing is known of its chemical nature it is first necessary to test for a number of substances having a common reaction and one that is easily recognizable rather than to test for a specific substance. Certain substances that fall naturally into a category of this kind are those that are easily oxidized, thereby acting as reducing agents. In this class of organic substance are included certain sugars that have an aldehyde or ketone group (1). When treated with the appropriate test reagent, reducing substances yield a colored and characteristic precipitate. Thus the present paper records the results of a microchemical investigation designed to detect a reducing substance in the intercellular spaces and intervening cell walls. It lays no claim to represent a finished piece of work, but is intended to show one method of testing for an intercellular substance and to suggest ways and means of identifying its chemical nature. The testing for other substances in these intercellular spaces is still in progress.

### Materials and Methods

Because of their narrow diameter, and the conspicuous development of intercellular spaces, seedling roots of white mustard (*Brassica alba* (L.) Boiss.) provide suitable material for this study. Seeds of this plant germinate readily between moist blotters at room temperature (22° C.). When the seedling roots reach a length of 5 to 10 mm. they are considered ready for treatment with the test reagent.

As mentioned above many of the best known test reagents are not satisfactory for a microchemical study of this kind. This is true for Fehling solution, Flückiger, and other reagents of a similar nature and all those that require strong heating. In every case, the reaction is so drastic as to render the meristematic tissue unsuitable for microscopic study. After considerable experimentation a modification of Tollen's reagent (12) appeared to be most

suitable for this purpose, since it is only slightly alkaline and does not require heating at a high temperature. It is prepared by adding concentrated ammonium hydroxide to a 3% solution of silver nitrate until the precipitate that forms just redissolves. According to Tuba *et al.* (13) silver nitrate is very easily reduced in ammoniacal solution, much less readily in neutral solution, and in acid solution with comparative difficulty.

Preliminary tests showed that the reagent penetrates the tissues rapidly and the length of time the material remains in the solution up to a maximum of 24 hr. appears to make little or no difference. The usual procedure is as follows: whole seedlings are immersed in the reagent in a test tube and placed in the dark for not longer than 18 hr. At the end of this time the seedlings have turned a dark brown color. They are then thoroughly washed in distilled water. After washing, but while still in water, the seedlings are exposed to full sunlight for four hours, either at room temperature or in a water bath at 37° C. The roots are then severed from the seedlings, dehydrated, embedded in paraffin, and cut transversely at 10  $\mu$  beginning at the extreme apex of the root. Since the sections are already stained brown by the reagent, no further staining is usually required. However, sections cut at 5  $\mu$  and stained with Delafield's haematoxylin are more suitable for cell wall study and for making photomicrographs.

## Results

As already pointed out (Cormack, 1947 (4) ) diamond-shaped intercellular spaces originate at an early stage in the meristem and become increasingly conspicuous at successively higher levels. In the present study of serial transverse sections they are first observable at a distance of 80 to 100  $\mu$  from the root apex. They are extremely small measuring 3 to 4  $\mu$  across their widest diameter. At this level the epidermal cells are all alike in size and shape and are scarcely distinguishable from the innermost row of root cap cells. Differentiation of the epidermis into long and short cells is first evident at about 120 to 150  $\mu$  above the root apex. By this time every section shows two well defined rows of diamond-shaped spaces in the cortex and occasionally a much smaller triangular-shaped space at the base of a short cell. This condition of the meristem taken at a somewhat higher level, approximately 250  $\mu$  above the root apex is clearly shown in Fig. 1. At this level the section is surrounded by two complete rows of small transparent root cap cells. Within the band of root cap tissue lies the single row of epidermal cells. The wedge-shaped cells each in contact with two cortical cells are the short cells, while the radially elongated cells situated between two short cells are the long cells. In the cortex the two rows of radially placed diamond-shaped spaces are prominent as are also a few triangular-shaped spaces in the epidermis. The cortical spaces are appreciably larger than at their origin and measure 7 to 9  $\mu$  across their widest diameter. While the cortical cells and short epidermal cells are densely filled with protoplasm and contain a prominent nucleus the long epidermal cells are beginning to show the first

signs of vacuolization. Differentiation of sieve tube elements in the stele is just beginning at this level.

Casual examination of transverse sections of roots treated with Tollen's reagent show that they are exactly comparable to those of control roots, killed and fixed with CRAF solution. It is thus evident that Tollen's reagent kills and fixes the meristematic tissue rapidly, without injury to the delicate cell walls and causing little or no plasmolysis. The condition of the meristem of a treated root at approximately  $175\ \mu$  from the root apex is shown in Fig. 2.

However, the most noteworthy feature in transverse sections of treated roots is the occurrence of small but well defined black stained bodies or particles in the intercellular spaces and aggregations of similar particles in the adjacent protoplasts. In general, they occur in all spaces throughout the meristematic region but they appear more conspicuously within a range extending from about  $150\ \mu$  to  $400\ \mu$  above the root apex and here they appear more numerous. Within this range some spaces contain more stained particles than others, but seldom, if ever, do they completely fill the whole space. When viewed with an oil immersion lens of sufficient resolving power these minute stainable particles are observed adhering to the surface of the walls forming the space and are observed also in the walls themselves. This feature is illustrated in the highly magnified photomicrographs, Figs. 3 and 4. Fig. 3 shows the condition of the cortex at approximately  $200\ \mu$  from the root apex. Here, fortunately, but quite by accident, the protoplasts of three radially placed cells have dropped out of the section, thus exposing the walls, which have been left intact. Numerous small black stained bodies are clearly visible in both spaces, and in the protoplasts. Fig. 4 represents a small portion of another section from the same root, but at a somewhat higher level. Some plasmolysis has occurred in these cells causing the protoplasts to draw away very slightly from the cell walls. Once again numerous small black stained particles are observable in the diamond-shaped space and in the thin cell walls.

It may be noted that no particles corresponding to those found after treatment with Tollen's reagent are observed in the intercellular spaces of untreated roots, whether the sections are stained with haematoxylin or not.

### Discussion

From the nutritional standpoint the position of actively dividing cells in relation to the food supply is a matter of considerable interest. Although the root meristem has been extensively studied by numerous investigators and the developmental stages clearly outlined in a recent review by Sifton (11), little is actually known concerning the movement of nutrients in this region. In this connection, Priestley (9) is of the firm belief that the "carbohydrate walls cleaving the meristematic mass are of the first importance, and are the channels along which these food materials reach the centres of protoplasmic synthesis". Also "that so long as the walls and intercellular spaces are filled with sap, there will be no difficulty in the transfer of solutes by this path to the actively growing cells". More recently Borgström (2) emphasizes the



FIGS. 1-4. Photomicrographs of transverse sections through the meristem of white mustard roots. FIG. 1,  $\times 130$ . Transverse section approximately  $250\ \mu$  from apex of untreated root, showing two rows of root cap cells, differentiation of the epidermis into short and long cells, and prominent diamond-shaped intercellular spaces in the cortex. FIG. 2,  $\times 300$ . One-half cross section of a treated root approximately  $175\ \mu$  from the root apex, showing marked plasmolysis in the two rows of root cap tissue, very slight plasmolysis in the epidermis and none in the cortical cells adjoining the spaces. FIG. 3,  $\times 2500$ . Transverse section about  $200\ \mu$  from apex, black-stained particles in two radially placed intercellular spaces and in the cell walls. In cutting, the protoplasts on one side of the spaces have dropped out of the section, but their walls forming the spaces are intact. FIG. 4,  $\times 2500$ . Transverse section of the same root at a slightly higher level showing a single space. Black-stained particles are conspicuous in the space and in the cell walls. The protoplasts have drawn slightly away from the cell walls.



importance of the cell walls in the transverse movement of auxins in plant tissues. From the results of numerous experiments he concludes that the walls in the transport direction must show a favored stretching.

The results of the present study demonstrate for the first time the occurrence of a substance in the intercellular spaces, and one capable of reducing alkaline silver. That this substance may be a reducing sugar seems highly probable, but since there may be a number of substances responsible for this reaction the solution of this particular aspect of the problem awaits further investigation.

The criticism might be raised that diffusion of the reducing substance may have occurred from the surrounding cells into intercellular spaces during the treatment. That this is the cause for the presence of a reducing substance in the intercellular spaces and intervening cell walls is quite unlikely. For at a level in the meristem where no plasmolysis occurs, particles are extremely numerous in all the spaces, while at somewhat higher levels where some plasmolysis occurs not a single particle is visible between the contracted protoplasm and the cell wall of individual cells forming the space.

The early development of intercellular spaces in the meristem of white mustard roots, somewhat previous to the differentiation of the epidermis into short and long cells, strongly suggests that from the very beginning they provide a more rapid movement of food to the outside. Although the detection of an intercellular substance strengthens the view that these radiating rows of spaces are the pathways for food movement, it does not prove this point. However, this assumption gives an intelligible explanation of the ability of the short epidermal cells to remain richly protoplasmic long after the long epidermal cells have become highly vacuolated. Moreover, as shown in roots of certain other plants, namely tomato and corn (4), where radiating rows of spaces either do not exist or do not extend completely through the cortex, the epidermis is not differentiated into long and short cells. In these roots all the epidermal cells are alike and they all begin to vacuolate at a point much closer to the root apex than do the short epidermal cells of white mustard roots.

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## NOTES ON SEED-BORNE FUNGI

### V. *CHAETOMIUM* SPECIES WITH DICHOTOMOUSLY BRANCHED HAIRS<sup>1</sup>

BY A. J. SKOLKO<sup>2</sup> AND J. W. GROVES<sup>3</sup>

#### Abstract

The large number of isolates of *Chaetomium* species obtained from agricultural seeds has made possible the critical study of some sections of the genus. In this paper the species with dichotomously branched hairs are considered. Of the six species previously recognized in this group, four have been isolated from seeds, *C. elatum* Kze. ex Fries, *C. dolichotrichum* Ames, *C. funiculum* Cooke, and *C. indicum* Corda. Two new species are described, *C. erectum* and *C. reflexum*, making a total of eight recognized species in this group.

#### The Genus *Chaetomium*

The genus *Chaetomium* was erected in 1817 by Kunze based upon *Chaetomium globosum* Kze. The present day concept of the genus includes those species with subglobose to elongate, colored, superficial perithecia that are provided with an ostiole. The perithecial wall is membranous and cellular and from the cells of the wall variously modified hairs are produced. The lateral hairs are frequently of different form from those borne at the summit of the perithecium. The terminal hairs may be spinelike, arched, flexed, coiled, circinate, or variously branched. Often secondary terminal hairs are developed with maturity and these may differ from the primary hairs. The asci are thin-walled and evanescent, usually clavate, sometimes cylindrical, typically eight-spored. The spores are simple, colored, typically lemon-shaped, and extruded through the ostiole either in an irregular mass or in cirrhi. Variations in spore shape range from globose to fusiform, triangular, or, in one case, square.

Since Kunze first established the genus, a large number of species have been described but many of these have been placed in synonymy or have been shown to be misdeterminations. Among the more important early contributions may be mentioned those of Zopf (1881 (18)), Palliser (1910 (12)), and Bainier (1910 (2)). In 1915 Chivers' monograph of the genus appeared and of the 114 species and 14 varieties that had previously been referred to the genus, he retained only 28, of which 11 were species described by him. Since Chivers' paper only a few new species have been added to the genus. Petrak (1915 (13)) described *C. fuscicolum*. Swift and Povah (Swift, 1929 (14)) described *C. subterraneum*, which Tschudy (1937 (16)) subsequently found

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to be identical with *C. globosum*. Garbowski (1936 (5)) established *C. tortuosum* and Tschudy (1937 (16)) described *C. ochraceum*, *C. cancroideum*, and *C. globosum* Kze. var. *affine*. Greathouse and Ames (1945 (6)) added *C. dolichotrichum*, *C. pachypodioides*, and *C. microcephalum*. Hughes (1946 (8)) described a species with four-spored asci, *C. tetrasporum*.

In the course of our examination of various commercial and registered agricultural seed stocks, members of the genus *Chaetomium* were amongst the commonest of the fungi isolated. The seed in all cases was first surface sterilized with a standard chlorine solution as described by Groves and Skolko (1944 (7)), and then plated on malt agar. It is by no means certain that the isolates of *Chaetomium* thus obtained are surface borne and capable of withstanding this sterilization or whether they are carried over in the seed as internal mycelium. Norris (1945 (10)) has claimed that *Chaetomium* spores are especially resistant to chlorine disinfection. Although *Chaetomium* species are usually regarded as being saprophytic and of particular economic importance as deteriorators of fabric, leather, and paper products, it has been observed that seed samples heavily infected with *Chaetomium* show a correspondingly low germination. The musty odor of these seed stocks is characteristic. It is frequently possible to distinguish cultures of *Chaetomium* by this odor even before any evidence of fruiting is visible.

There are few records of *Chaetomium* species isolated from seed. Garbowski (1936 (5)) lists *C. globosum*, *C. spirale*, and *C. tortuosum* isolated from seed of *Pinus sylvestris*. Orton's bibliography (1931 (11)) of seed-borne parasites lists *Chaetomium* sp. as being isolated from seeds of corn (*Zea Mays*) and from wheat (*Triticum aestivum*). Tullis (1936 (17)) isolated *Chaetomium* spp. from discolored rice kernels. Chilton (1942 (3)) reported the occurrence of *Chaetomium* sp. in seed of clover. Tervet (1945 (15)) found *Chaetomium* sp. on soybeans, and Arndt (1946 (1)) found a species of *Chaetomium* on cotton seed. We have isolated some 15 species from a variety of vegetable and field crop seeds; of these four are considered to be new. Although the pathological significance of these species still remains obscure, the large number of isolates has provided an opportunity for a critical study of some sections of the genus and has provided many new records.

It has been possible to examine a number of the exsiccati cited by Chivers. For the most part the exsiccati in the mycological herbarium of the Dominion Department of Agriculture, Division of Botany and Plant Pathology, Ottawa, Canada, (DAOM), agree with those examined by Chivers. In a number of cases it is obvious that the original collection was a mixed one. This has undoubtedly added to the confusion. Examples of such instances are noted in the discussion of the various species. Type or authentic material of most valid species has been examined, and although less than half of the recognized species of *Chaetomium* have been isolated from seed, since it has been possible to examine most of the types, a key to the genus has been prepared to include

the more recent species. In this paper the key to those species with dichotomously branched hairs is presented. Full descriptions are given only for species that we have isolated from seed.

Reference is frequently made in descriptions of species of *Chaetomium* to terminal or lateral hairs with elongated, hyaline tips that appear collapsed. It has been observed that in freshly made mounts of a number of such species these hyaline tips are greatly inflated, suggesting that under favorable conditions of humidity this is the normal appearance but when placed in a mounting medium the inflated tips collapse. An irregular opening may frequently be observed in the wall of the hair just below the narrow portion of the tip, which is suggestive of excessive internal pressure. Examination of unmounted perithecia by reflected light often discloses sparkling tips on the hairs. This may in part be a result of the refractive nature of the inflated tips and in part to the hyaline character of the tip alone. It is possible that the irregular crystalline projections on the hairs may be responsible for the reflection of light from the tips for it is sometimes found that these crystals are most numerous toward the tip.

Little use has been made of the character of the ascus in the separation of species. The difficulty of observing the early evanescent asci makes them of doubtful value in this connection.

Cultural characters referred to in the following descriptions apply to the appearance of the colony on 2% malt agar plates kept at room temperature in diffuse light. Most cultures have been grown on filter paper and have been deposited in the herbarium of the Division of Botany and Plant Pathology at Ottawa.

Prior to 1937 only four species with dichotomously branched hairs were recognized, *C. elatum* Kze. ex Fries, *C. indicum* Cda., *C. funiculum* Cke., and *C. cuniculorum* Fckl. *C. elatum* and *C. cuniculorum* remain sufficiently distinct although the latter species appears to be less common than early reports suggest and hence is not too well understood. The two other species, *C. indicum* and *C. funiculum*, have obviously presented difficulties in their separation. This is perhaps understandable in view of the numerous intermediate forms that exist and the fact that new species have since been segregated from what formerly, in all likelihood, would have been considered one or other of these two species. Since 1937 two such species have been recognized and in the present paper two others are added. They differ essentially in the presence or absence of terminal, unbranched hairs, in the character of the dichotomously branched hairs, and in the size of the perithecium. At one end of the series is *C. funiculum* with simple terminal hairs and dichotomously branched hairs with the branches at acute angles, roughened, and with more or less straight, short internodes. Close to this species is *C. dolichotrichum* in which the hairs are more graceful, with long tips, and with the branches of the dichotomous hairs almost at right angles to the axis, smooth or nearly so, usually slightly reflexed, with long internodes. The remaining species either lack simple terminal hairs or, if present, they soon

become obscured. *C. indicum* is rather larger than the others, with regular and repeatedly dichotomously branched hairs, the branches arising at right angles to the axis and forming a regular network; *C. erectum* with rigid, erect hairs and few, short, blunt branches; *C. reflexum* with short, stout, arcuate or curved hairs with the short, blunt branches strongly recurved; and *C. cancroideum* with graceful, ascending, terminal hairs, frequently dichotomously branched with the branches arising at an acute angle and incurved to give a 'crab claw' appearance. In culture these species differ widely and within some of the species there are sufficient cultural differences to suggest that further separations may be expected.

### KEY TO THE SPECIES OF *CHAETOMIUM* WITH DICHOTOMOUSLY BRANCHED HAIRS

- A. Branches of terminal hairs essentially straight.
  - B. Unbranched terminal hairs present at maturity (in addition to dichotomously branched hairs), conspicuous, usually projecting beyond the mass of branched hairs.
    - C. Perithecia large, up to  $450\mu$  wide; terminal hairs coarsely roughened, stout,  $5-8\mu$  wide; spores lemon-shaped, colored, large,  $10-11 \times 7-8\mu$ .....*C. elatum*
    - CC. Perithecia medium size,  $370\mu$ ; branches of terminal hairs anastomosing; spores elliptical or spindle-shaped,  $10-12 \times 7-9\mu$ .....*C. cuniculorum*
    - CCC. Perithecia small, not exceeding  $200\mu$  in width; spores oval to ovoid, less than  $7\mu$  long.
    - D. Branched hairs smooth or slightly roughened, branches few with long internodes, widespreading and sometimes slightly reflexed; unbranched hairs graceful, long projecting, tapering gradually to a long tip.....*C. dolichotrichum*
    - DD. Branched hairs coarse, often with rough projections, branches at acute angle and with short internodes; unbranched hairs rigid with rapidly tapering tip, projecting only a short distance beyond the mass of branched hairs.....*C. funiculum*
  - BB. Unbranched terminal hairs absent or obscure at maturity (may be present in younger stages).
    - C. Perithecia up to  $200\mu$  wide; terminal hairs repeatedly and regularly branched forming a close network, hairs roughened by numerous blunt projections....*C. indicum*
    - CC. Perithecia not more than  $140\mu$  wide; terminal hairs rigid with only a few short branches near the top, internodes short, hairs smooth with few sharp projections at the blunt, hyaline tip.....*C. erectum*
- AA. Branches of terminal hairs regularly curved.
  - B. Terminal hairs straight with incurved branches, producing characteristic 'crab claw' effect; perithecia  $175\mu$  wide.....*C. cancroideum*
  - BB. Terminal hairs arcuate with branches strongly reflexed, perithecia not over  $125\mu$  wide.....*C. reflexum*

***Chaetomium elatum* Kze. ex Fries (Plate I) Deutsch. Schw. 8 : 3. 1818.**

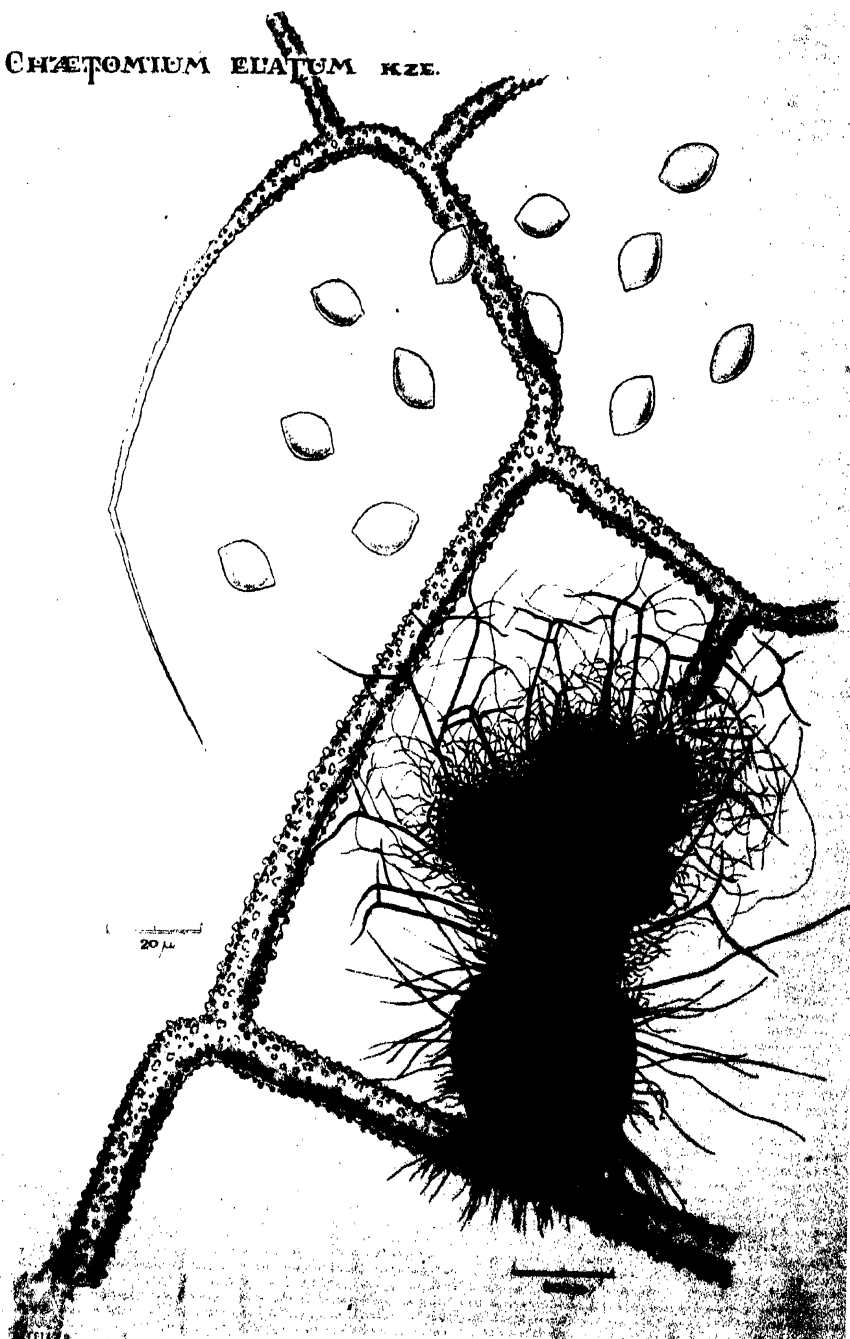
Culture at first white, becoming yellowish, with much aerial mycelium.

Perithecia black, subglobose or ovoid, rather large, up to  $500\mu$  long and  $450\mu$  wide, produced late, erratic, and frequently covered by white aerial mycelium.

Lateral hairs unbranched, straight or slightly flexed, roughened by irregular projections, about  $4\mu$  wide at base, ending in a blunt rounded tip.

Terminal hairs: simple, unbranched hairs present. Branched hairs coarsely roughened, dark olive-brown, stout,  $5-8\mu$  wide, several times

CHÆTOMIUM ELATUM KZE.





dichotomously branched (the branching is not strictly dichotomous in that one branch arises slightly below the other and the main axis continues through making a right-angled turn to form a second branch). Secondary hairs similar to the primary except much more narrow and not so dark and with the branching less regular. Sometimes the secondary hairs become alternately branched at right angles to the axis. Both lateral and terminal hairs arise from a rosette of cells of the perithecial wall.

Spores lemon-shaped, dark olive, umbonate at both ends,  $11-13 \times 9-9.5\mu$ .

The large perithecia together with the broad, coarsely roughened hairs, distinctive branching, and large, lemon-shaped spores make this species easily distinguishable.

*Chaetomium elatum* has been isolated from seeds of *Apium graveolens* L. var. *dulce* DC. (celery), *Beta vulgaris* L. (beets), *B. vulgaris* L. var. *cicla* L. (Swiss chard), *Cucurbita Pepo* L. (pumpkin), *Daucus carota* L. var. *sativa* DC. (carrot), *Medicago sativa* L. (alfalfa), *Petroselinum hortense* Hoffm. (parsley), *Pisum sativum* L. (peas), *Raphanus sativus* L. (radish), and *Trifolium hybridum* L. (alsike).

It has been isolated from seeds from Ontario, Manitoba, Saskatchewan, and British Columbia in Canada, and from New Jersey, Connecticut, Pennsylvania, Michigan, Washington, and California in the United States.

The citation for the name *Chaetomium elatum* presented some difficulty owing to the fact that the name was cited differently by different authors and the early literature was not readily available. Through the kindness of Dr. S. P. Wiltshire and Dr. G. R. Bisby it was possible to have the early literature examined. They informed us regarding *C. elatum* as follows:

"*C. elatum* was described in the folder accompanying Lief. 8 of Schmidt and Kunze, Deutschl. Schwämme, 1818, as follows: 'CLXXXIV *Chaetomium elatum*: sporangio subturbinato, pilis circa basin simplicibus strictis, in superficie rigidis ramosis implexis longissimis sporidiis oviformi-globosis. G. Kunze monographia hujus generis adhuc inedita.' From the last sentence, and from the fact that the specimen of No. 184 is labelled '*Chaetomium elatum* Kunze', it is evident that the species was *C. elatum* Kunze apud Schmidt & Kunze; now it is *C. elatum* Kunze ex Fr. The sentence quoted also makes it evident that one can follow Fries and cite the genus as *Chaetomium* Kunze ex Fr."

*Exsiccati*.—Ell. & Ev. Fung. Columb. 621; de Jacz. fung. Rossiae 83; Roum. Fung. Sel. Gall. 1428.

Sub *C. atrum* Lk. var. *distinctum* Roum. (syn. *C. elatum* Kze.): Roum. Fung. Sel. Gall. 3883.

Sub *C. chartarum* Ehr. (syn. *C. globosum* Kze.): Roum. Fung. Sel. Gall. 1090. Chivers found only *Stachybotrys lobulata*. Our specimen shows abundant and typical material of *C. elatum* Kze. only.

Sub *C. comatum* (Tode) Fr. (syn. *C. elatum* Kze.): Roum. Fung. Sel. Gall. 975; D. Sacc. Myc. Ital. 841.



Sub *C. Libertii* Roum. et Pat. (syn. *C. elatum* Kze.): Roum. Fung. Sel. Gall. 2376.

Sub *C. pannosum* Wallr. (syn. *C. elatum* Kze.): Roum. Fung. Sel. Gall. 61; a collection in the mycological herbarium at Ottawa labelled "*Chaetomium pannosum* Wallr., auf Carex, leg. P. Sydow, 1898", has also been found to be *C. elatum* Kze.

*C. olivaceum* Cke. & Ellis is considered to be a synonym of *C. globosum* Kze. by Chivers. Ellis and Everhart Fung. Columb. 512, under *C. olivaceum*, was found by Chivers to consist in part of *C. globosum* Kze., of *C. elatum* Kze., and of *Chaetomidium fimeti* (Fckl.) Zopf. Our examination of this exsiccatum revealed only young perithecia and spores of what is likely *C. funiculum* Cke.

*C. olivaceum* Cke. & Ellis f. *chartarum* Roum., a synonym of *C. globosum* Kze., is listed by Chivers under *C. globosum* in Roum. Fung. Sel. Gall. 4930. Our examination of Fung. Gall. 4930 yielded *C. elatum* Kze. and what appeared to be young perithecia of *C. cochliodes* Palliser.

In D. Sacc. Myc. Ital. 1475, sub *C. pannosum* Wallr. we have found only *C. murorum* Cda. whereas Chivers found both *C. elatum* Kze. and *C. murorum* Cda.

In Roum. Fung. Sel. Gall. 325, sub *C. atrum* Lk. (syn. *C. elatum* Kze.) only *Chaetomidium fimeti* (Fckl.) Zopf could be found, as also reported by Chivers.

In Roum. Fung. Sel. Gall. 2496, sub *C. atrum* Lk. f. *Therryana* Roum. et Pat. we have found both *C. elatum* Kze. and *Chaetomidium fimeti* (Fckl.) Zopf. Chivers considers *C. atrum* f. *Therryana* to be a synonym of *Chaetomidium fimeti* and not of *C. elatum* on the basis of "a study of the original description and accompanying figures (Rev. Myc. 5 : 29) together with an examination of authentic specimens in Fung. Gall. No. 2496".

In E. & E. Fung. Columb. XI, 1034, we have found young perithecia and spores of *C. funiculum* Cke. only, whereas Chivers cites this specimen under *C. elatum* Kze. without further comment.

***Chaetomium cuniculorum*** Fuckel Symb. Myc. p. 89. 1870.

This species has not been isolated from seed nor has it been possible to examine any satisfactory specimens. A culture so-named received from Centraalbureau voor Schimmelcultures failed to fruit.

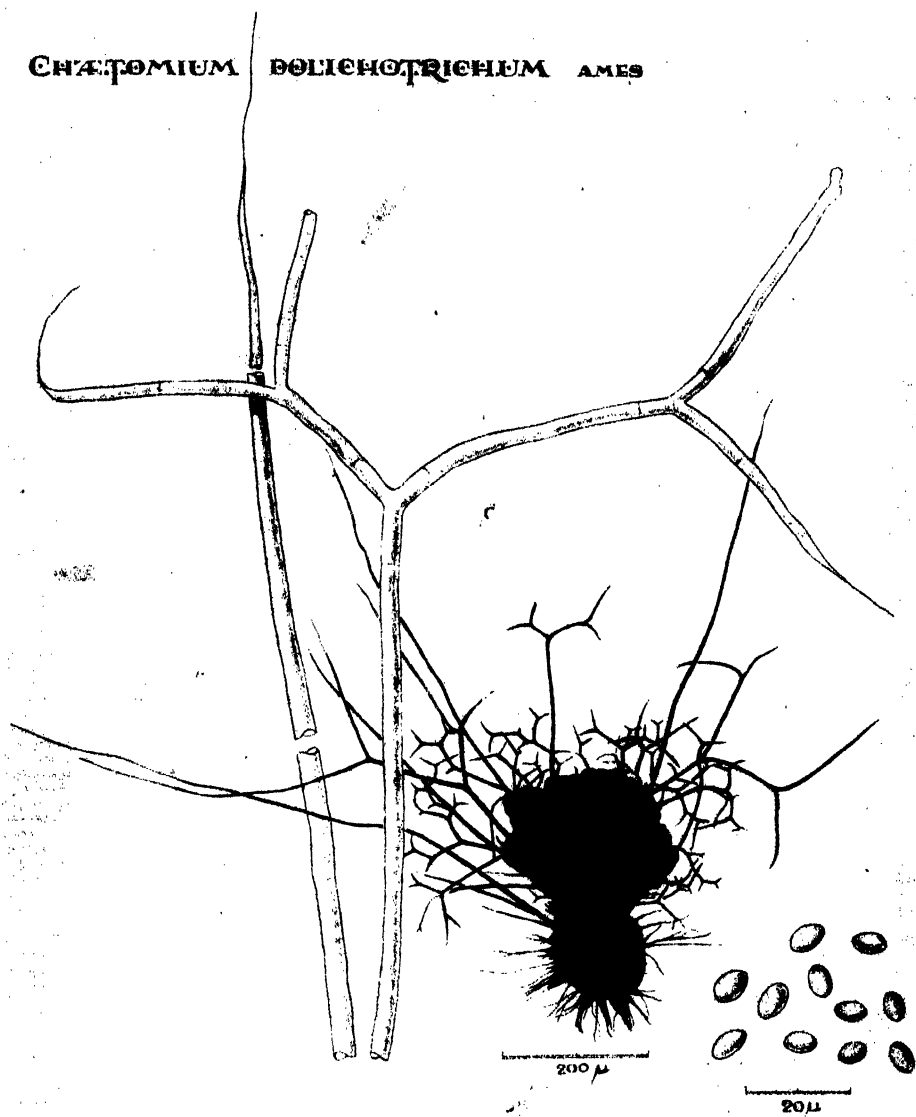
This species is described as having dichotomously branched terminal hairs but should be easily distinguishable by the large size of the perithecium, 370 $\mu$ , stiff, stout hairs with anastomosing dichotomous branches, simple terminal hairs, and large, elliptical or spindle-shaped spores, 10-12  $\times$  7-9 $\mu$ . In these characters it would appear to most nearly approach *C. elatum*, which, however, has larger perithecia, and lemon-shaped, broader spores.

***Chaetomium dolichotrichum*** Ames (Plate II) Mycologia, 37 : 145. 1945.

Culture at first white to gray becoming smoky olive with perithecial formation.

Perithecia olive to smoky-gray colored, globose to subglobose, 100-150 $\mu$  in diameter, produced early, at the center of the culture.

**CHARTOMIUM BOLICHOTRICHUM AMES**





Lateral hairs few, relatively short, brown becoming lighter colored toward the tip, about  $3-4\mu$  wide at base, smooth to slightly roughened, regularly septate, tapering abruptly to a blunt round point or in some cases with long collapsed tip.

Primary terminal hairs varying from extremely long, unbranched to once dichotomously branched to several times regularly and divergently dichotomously branched (the more frequently branched, the shorter the extension above the mass of secondary hairs and spores), smooth or only slightly roughened or with a few prominent blunt projections, dark brown, regularly septate,  $5.5\mu$  wide at base; branches widely divergent, often at right angles to the axis, sometimes slightly reflexed.

Secondary terminal hairs forming a compact mass of dichotomously branched hairs about the base of the primary hairs, light brown colored, septate, minutely roughened, of irregular width, varying from  $2.5$  to  $3\mu$  wide.

Spores light brown, oval to ovoid,  $6.0 \times 4.0-5.5 \mu$ .

This species is intermediate between *C. indicum* and *C. funiculum* but it is distinguishable because of its distinctive primary hairs as described above and by the appearance of the culture. Chivers did not recognize this species as distinct from *C. indicum*. He identified a specimen collected by Thaxter in 1908 from a Brazil nut culture as *C. indicum* but examination of this specimen discloses it to be typical of *C. dolichotrichum*. *C. dolichotrichum* was described by Ames (1945 (6)) and examination of both the type specimen and type culture shows that it is identical with a number of isolations from seed that had been segregated as a new species prior to Ames' publication.

*Chaetomium dolichotrichum* has been isolated from seeds of *Linum usitatissimum* L. (flax), *Pisum sativum* L. (peas), and *Raphanus sativus* L. (radish).

It has been isolated from seeds from Quebec, Ontario, Saskatchewan, and Connecticut.

***Chaetomium funiculum*** Cooke (Plate III) Grev. 1 : 176. 1873.

Culture at first gray, becoming greenish.

Perithecia dark green, subglobose,  $140-185\mu$  wide.

Lateral hairs numerous, unbranched, long, straight to slightly curved, dark brown becoming lighter toward the long collapsed tip, septate to obscurely septate, smooth to finely roughened by blunt projections,  $4-5\mu$  wide at the base.

Terminal hairs of two types; unbranched hairs similar to the lateral hairs, at maturity projecting a short distance or almost obscured by the spore mass and branched hairs; dichotomously branched hairs, dark brown, coarsely roughened by blunt projections and with the internodes straight, sometimes slightly curved, branches at an acute angle, later formed hairs becoming less regularly branched, narrow, constricted at the septa, finely roughened, at length forming a closely branched network of slightly roughened branches; the whole forming a compact dark mass with the spores above the perithecium.

Spores ovoid, oval to elliptical, indistinctly umbonate to acute on the ends, colored,  $5.0-6.5 \times 4.5-5.0\mu$ .

This is a variable species and one that may be easily confused with *C. indicum* on the one hand, and with *C. dolichotrichum* on the other. It may be distinguished from *C. indicum* by the presence of unbranched terminal hairs and the less regular and acute branching of the dichotomously branched terminal hairs. It differs from *C. dolichotrichum* in its straight, short projecting, unbranched terminal hairs and the stout, straight, roughened, acute branches of the branched terminal hairs.

*Chaetomium funiculum* has been isolated from seeds of *Allium Cepa* L. (onion), *Brassica oleracea* L. var. *capitata* L. (cabbage), *B. Rapa* L. (turnip), *Cucumis sativa* L. (cucumber), *Festuca elatior* L. (meadow fescue), *Glycine Max* Merr. (soybean), *Linum usitatissimum* L. (flax), *Lycopersicon esculentum* Mill. (tomato), *Pisum sativum* L. (peas), *Vicia villosa* Roth. (hairy vetch), and *Zea Mays* L. (corn).

It has been isolated from seeds from Quebec, Ontario, Manitoba, British Columbia, and Michigan in North America, and also from Denmark and Japan.

*Exsiccati*.—Sub *Chaetomium elatum* Kze.: E. & E. Fung. Columb. 1034, we have found *C. funiculum* whereas Chivers lists this under *C. elatum*. Sub *C. olivaceum* Cke. & Ellis (syn. *C. globosum* Kze.): E. & E. Fung. Columb. 512, we found only *C. funiculum*. Chivers states that this exsiccatum is a mixture of *C. globosum*, *C. elatum*, and *Chaetomidium fimeti*. Sub *C. setosum* E. & E. (syn. *C. funiculum* Cke.): E. & E. Fung. Columb. 1126. The type of *C. setosum* E. & E. in N.A.F. 2d Ser. XXXV, 3423, has been examined and although the perithecia are immature the specimen agrees with *C. funiculum*.

***Chaetomium indicum*** Corda (Plate IV) Icon. Fung. IV, t. VIII, f. 104. 1840.

Culture variable in color and rate of growth, varying from gray, yellowish to green.

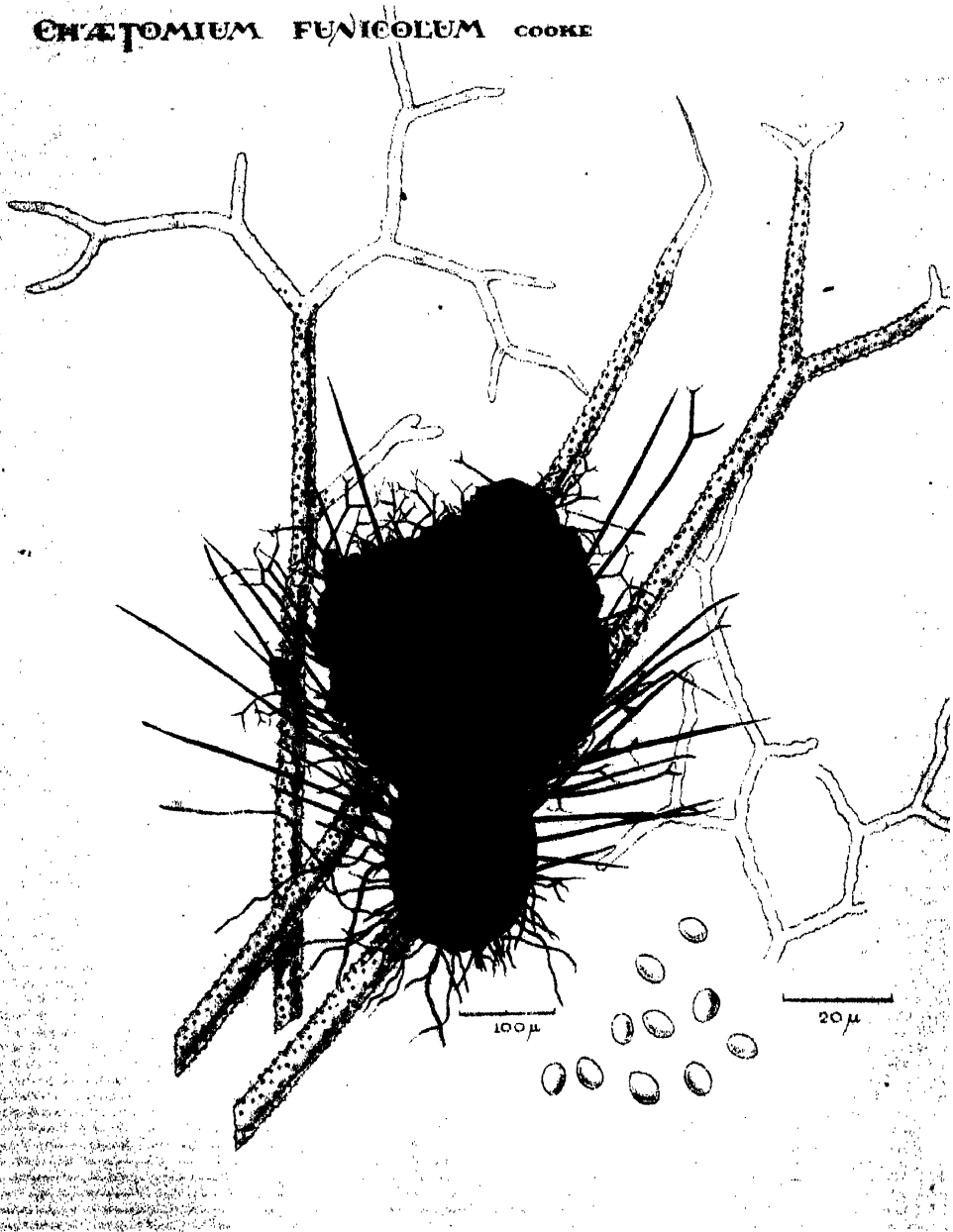
Perithecia olive-green, subglobose, 120–170–(200) $\mu$  wide.

Lateral hairs lacking or few, unbranched or few times dichotomously branched, straight or slightly curved upward, dark brown below becoming lightly colored above, tapering to a long, collapsed hyaline tip, regularly septate, smooth or very slightly roughened.

Terminal hairs: unbranched hairs lacking or few and obscured by the mass of branched hairs and spores; branched hairs several times dichotomously and regularly branched almost at right angles to the axis and slightly reflexed (sometimes slightly incurved) with the internodes of approximately equal length, the whole forming a regular network, dark brown, slightly roughened by rounded projections near the blunt tips, 5–6 $\mu$  wide; later formed hairs narrow, branching acutely, uneven, roughened by numerous sharp projections.

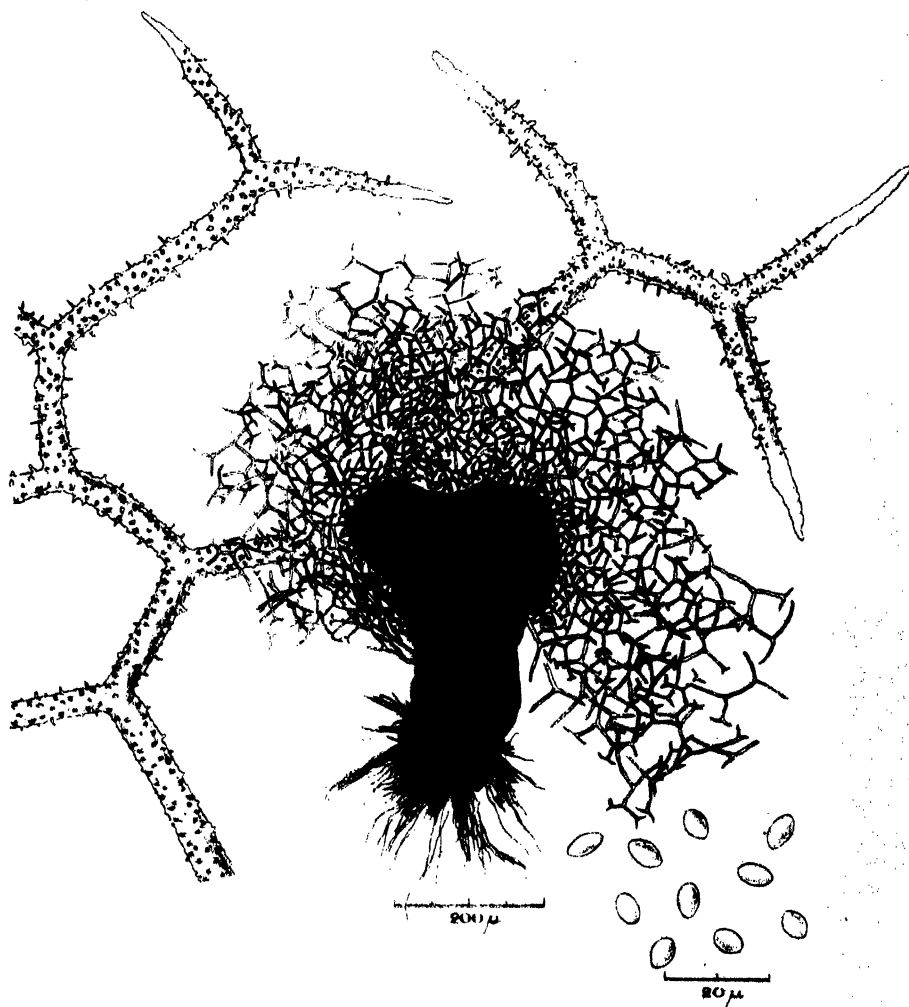
Spores ovoid, oval to broadly oval to subglobose, sometimes flattened on one side, rounded on the ends or slightly umbonate at one or both ends, dark colored (5.0)–6.5–7.5  $\times$  (4.0)–4.5–5.5 $\mu$ .

**CHAETOMIUM FUNICULUM COOKE**





CHÆTOMIUM INHIBICUM CORBA







The above description applies, in the main, to typical material of *C. indicum* but in many specimens intermediate characters between *C. indicum* and *C. funiculum* may be encountered. The two species are obviously closely related with many intermediate and borderline forms. In some instances assigning a specimen to one or other of these two species has been made with some misgivings. Typical specimens of *C. indicum* differ essentially in the absence of unbranched lateral and terminal hairs (except in the very early stages of perithecial development), and in the regularly dichotomous branching of the terminal hairs, the branches widespread and frequently slightly reflexed. In *C. funiculum* the unbranched lateral hairs are numerous and the unbranched terminal hairs are persistent, while the dichotomously branched terminal hairs have the branches less regularly disposed and at acute angles to the axis. Obviously the variations that occur within these two species have been responsible for much confusion as indicated by the work of Chivers. Two species that undoubtedly would formerly have been considered as *C. indicum* have already been described, namely, *C. dolichotrichum* Ames and *C. cancroideum* Tschudy. In the present paper two other related species have been distinguished and described as new, *C. reflexum* and *C. erectum*.

Two specimens identified by Chivers as *C. indicum* have been examined. One of these, from a laboratory culture at Cambridge, Mass., was found to be typical. The other, collected by R. Thaxter from a Brazil nut, was found to be *C. dolichotrichum* Ames. A culture received from the Centraalbureau voor Schimmelcultures and labelled *C. indicum* Cda. was found to be *C. cancroideum* Tschudy.

*Chaetomium indicum* has been isolated from the following seeds: *Beta vulgaris* L. var. *cicla* L. (Swiss chard), *Daucus carota* L. var. *sativa* DC. (carrot), *Linum usitatissimum* L. (flax), *Lolium perenne* L. (rye grass), *Lycopersicon esculentum* Mill. (tomato), *Phaseolus vulgaris* L. (beans), *Pisum sativum* L. (peas), *Taraxacum kok-saghyz* Rod. (Russian dandelion).

It has been isolated from seeds from Nova Scotia, Quebec, Ontario, British Columbia, New Jersey, and Minnesota in North America, and also from Scotland and Holland.

***Chaetomium erectum* n. sp. (Plate V)**

Culture consisting largely of closely grouped perithecia, dark green in color, with a narrow white to yellowish margin.

Perithecia dark green, ovoid, 130–140 $\mu$  wide, with numerous dark rhizoids.

Lateral hairs simple, few, relatively short, 4 $\mu$  wide at base, septate, colored below becoming hyaline above, tip long, collapsed.

Terminal hairs: unbranched hairs lacking; branched hairs few times dichotomously branched, stout, 6.5 $\mu$  wide, rigid, dark, smooth below, becoming hyaline and somewhat roughened above especially toward the short tip, internodes short, the hairs forming an erect, relatively short, stout cluster at the top of the perithecium.

Spores dark, unequally oval to elliptical, acute at both ends,  $6.5-7.5 \times 4.5-5.0\mu$ .

Peritheciis atro-viridis, ovoideis,  $130-140\mu$  latis; rhizoideis numerosis, brunneis; pilis lateralibus paucis, simplicibus, septatis, basi  $4\mu$  diam., brunneis, apice hyalinis, collapsis; pilis terminalibus dichotome paululum ramosis, rigidis, atro-brunneis, basi  $6.5\mu$  diam. lenibus, ultimis ramis hyalinis asperulis, brevibus; ascosporis olivaceo-brunneis, inaequaliter ovoideis vel ellipticis, utrinque acutis,  $6.5-7.5 \times 4.5-5.0\mu$ .

Hab.: *Petroselinum hortense* Hoffm.—semina germinantes.

Type specimen has been deposited in the herbarium at Ottawa as number DAOM 14205.

*C. erectum* has been isolated only once, from seed of parsley, from Milford, Conn., July 6, 1943. Culture 42-I-351A.

This species may be confused with the early stages of *C. indicum*, which, however, produces many times branched hairs that are less rigid and erect and with longer internodes, the branching nearly at right angles. It resembles *C. reflexum*, which also has relatively short, stout, branched terminal hairs but in *C. reflexum* the hairs are arcuate and the branches strongly reflexed or curved.

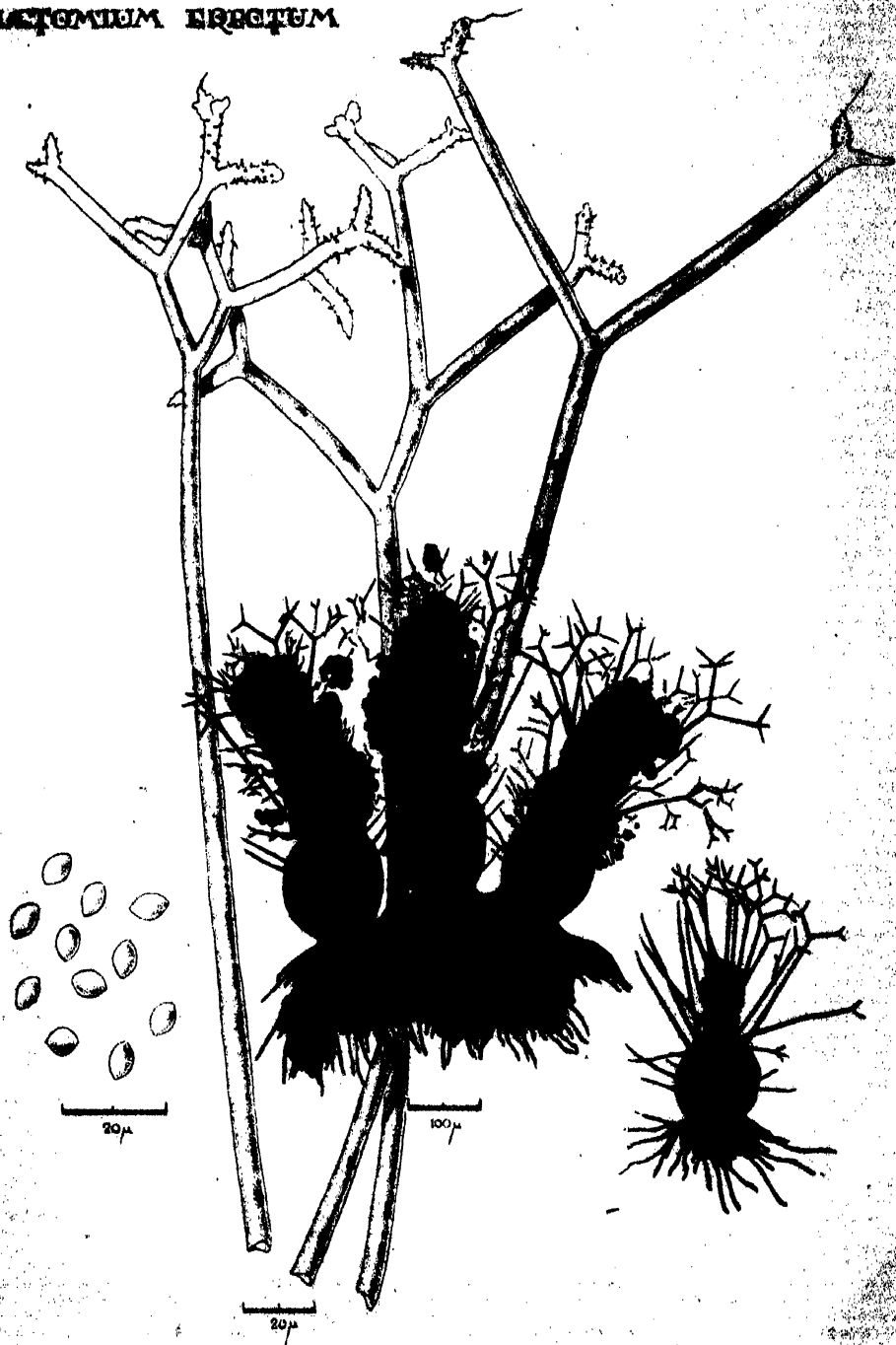
***Chaetomium cancroideum*** Tschudy (Plate VI) Am. J. Botany, 24 : 478. 1937.

Although this species has not been isolated from seed it has been possible to examine three specimens, one in the Farlow Herbarium identified by L. M. Ames, 1944, and two cultures from the Centraalbureau voor Schimmelcultures, one of which was incorrectly labelled *C. indicum* Cda.

The original description as presented by Tschudy follows in part: "... Perithecia small, subglobose,  $180 \times 160\mu$  ( $105-200 \times 101-175$ ), attached to substratum by rhizoids.... Lateral hairs not prominent, mostly smooth, stiff, dark brown at base becoming lighter brown to hyaline at tip, rarely and obscurely septate; terminal hairs forming an extremely dense compact head, dichotomously branched in the upper half of the hair; branches very many; angles between branches narrow, acute, concave on the inner side and thus producing the characteristic overlapping or 'crab-claw' effect; occasionally inflated and constricted while the entire length is covered with spines and irregular projections; terminal perithecial spines medium long, brown to black in color, and usually inconspicuous and hidden by the profuse dichotomous setae..... Spores when young greenish blue, with one to several refractive globules; when mature dark brown, lemon shaped, ends more often rounded than apiculate or umbonate;  $5.1 \times 3.3\mu$  ( $4.4-5.8 \times 3.2-4.5$ );.....".

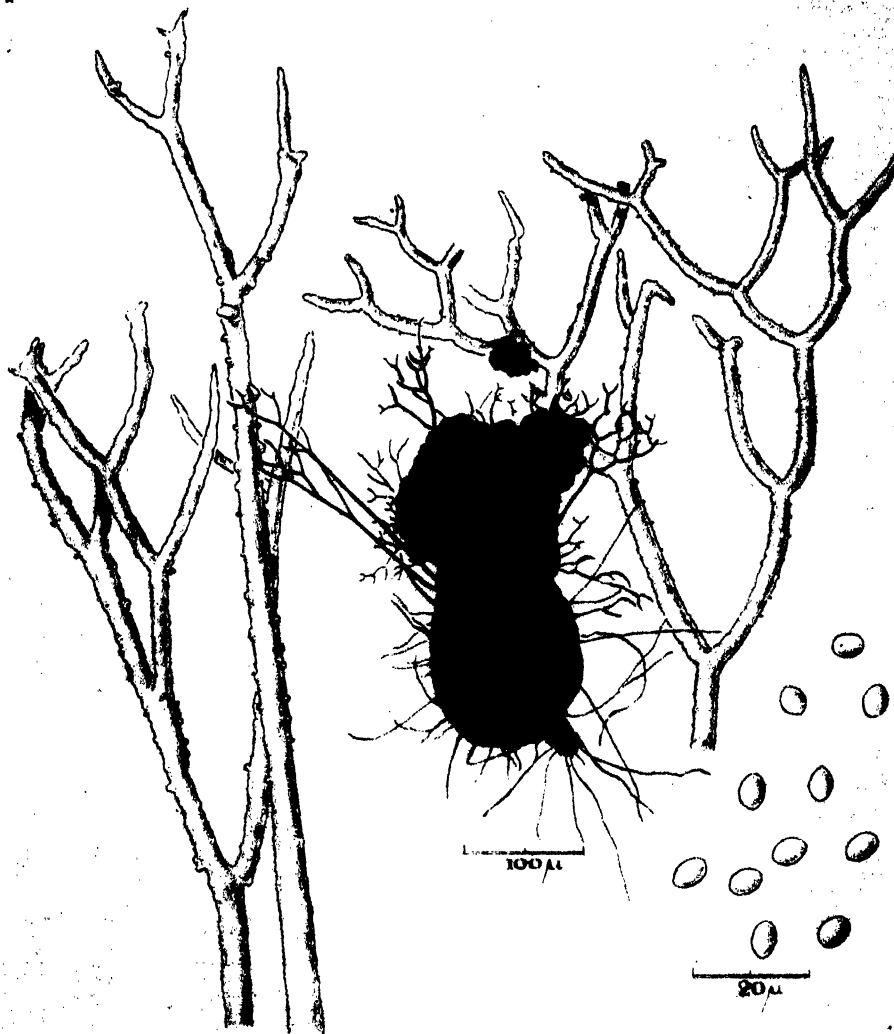
This species may be distinguished from *C. erectum* and *C. reflexum*, which also have small perithecia and dichotomously branched terminal hairs, by the fact that the terminal hairs are more graceful with numerous branches arising at acute angles and concave or incurved producing the so-called 'crab claw' effect.

**ONATOMIUM ERIGTUM**





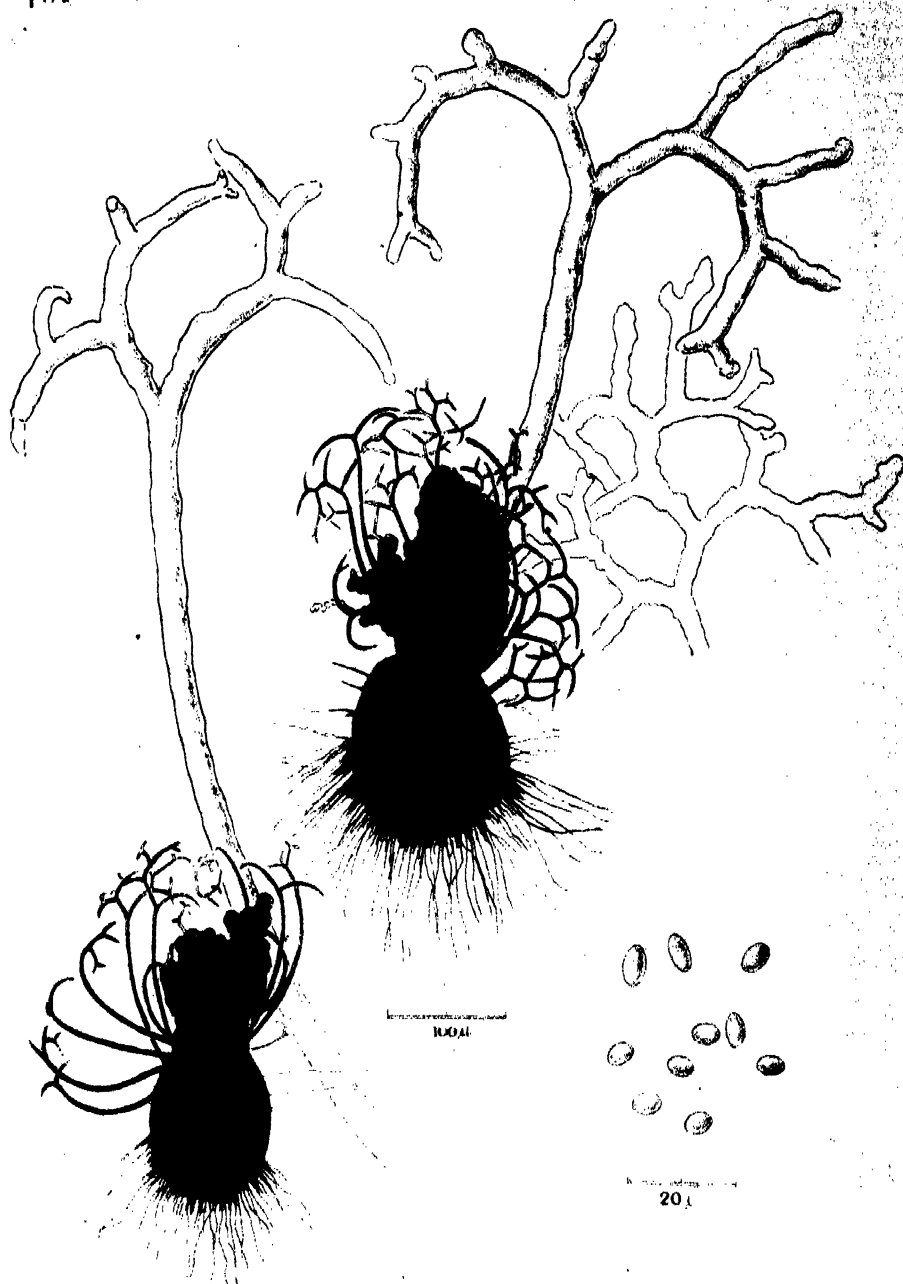
*CHETOMIUM CANEROIDEUM* Tsch.





# CHÆTOMIUM REFLEXUM

TYPE



Stromata on wood of *Quercus*  
1000x

Spores of *Chætomium reflexum*  
20x





***Chaetomium reflexum* n. sp. (Plate VII)**

Culture at first light gray in color, becoming dark gray with the formation of perithecia.

Perithecia black, ovoid to subglobose, small, 100–125 $\mu$  wide, closely arranged forming an almost continuous layer, obscured by the dense mass of dark rhizoids and terminal hairs.

Lateral hairs few, unbranched, very short, narrow, 2.5 $\mu$  wide at base, uneven, only slightly colored, with rounded tip, appearing as modified, short rhizoids.

Terminal hairs short, stout, 5 $\mu$  wide, arcuate from the base, with short, stout, strongly recurved, dichotomous branches, tips blunt, dark brown, only slightly roughened above, obscurely septate, forming a dense, flat-topped mass above the perithecium.

Spores light olive-brown, oval, slightly flattened on one side, rounded to slightly acute on the ends, 5.0–6.5  $\times$  4.0–4.5 $\mu$ .

Peritheciis, atris, ovoideis vel subglobosis, 100–125 $\mu$  diam.; rhizoideis numerosis, brunneis; pilis lateralibus paucis, simplicibus, brevissimis, subhyalinis, basi 2.5 $\mu$  diam., apice obtusis; pilis terminalibus, brevibus, 5 $\mu$  diam., arcuatis, dichotome ramosis, apice forte recurvatis, obtusis, brunneis, minute spiculis, obscure septatis; ascosporis olivaceo-brunneis, ovoideis, leviter inaequilateris, utrinque rotundatis vel subacutis, 5.0–6.5  $\times$  4.0–4.5 $\mu$ .

Hab.: *Lycopersicon*, *Pisum*, *Hibiscus*, *Capsicum*—semina germinantes.

The type specimen, from *Capsicum annuum* L., has been deposited in the herbarium at Ottawa under number DAOM 14201.

*Chaetomium reflexum* has been isolated from seeds of *Capsicum annuum* L. (pepper), *Hibiscus esculentus* L. (okra), *Lycopersicon esculentum* Mill. (tomato), and *Pisum sativum* L. (peas).

It has been isolated from seeds from Manitoba, New Jersey, and Ohio.

*C. reflexum* may be distinguished by the small, closely arranged perithecia, the flat-topped mass of short, stout, curved terminal hairs with reflexed, short, blunt branches. Cf. *C. erectum* and *C. cancroideum*.

### Acknowledgments

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## STUDIES IN FOREST PATHOLOGY

### VI. IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI<sup>1</sup>

By MILDRED K. NOBLES<sup>2</sup>

#### Abstract

A numerical key for the identification of cultures of 126 species of wood-rotting fungi, with accompanying descriptions of the cultural characters of each species, is presented. The list of species includes many of the fungi most frequently encountered in studies of decay in various hosts, the species having been chosen without restriction as to host or locality. The descriptions and key have been based on cultural characters, both macroscopic and microscopic, that can be demonstrated readily without the aid of elaborate equipment or methods. Thus, cultures were grown in Petri dishes on Difco malt agar and on malt agar to which gallic or tannic acid had been added. They were incubated in the dark at room temperature and examined at weekly intervals for six weeks. Host relationships and physiological and morphological characters proved to be of diagnostic value. The physiological characters used in the key include the pigmentation of the mycelial mat, color changes induced in the agar by the fungus, rate of growth, and effect on media containing gallic or tannic acid. Morphological characters used in diagnosis were types and septation of hyphae, occurrence of secondary spores—chlamydospores, conidia, oidia—and of special structures, such as cystidia and setae, and the formation of fruit bodies. The numerical key will permit the interpolation of additional species as they are described and their code numbers prepared.

#### Table of Contents

	PAGE
Introduction.....	282
Review of literature.....	283
Cultures.....	285
Methods.....	285
Glossary of descriptive terms.....	287
Key for the identification of cultures.....	290
Descriptions of cultures.....	313
Discussion.....	405
Acknowledgments.....	406
References.....	406
Index.....	412
Plates.....after page	414

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## Introduction

The peculiar difficulty attached to the identification of cultures of wood-rotting fungi lies in the fact that, although they belong to species that have already been classified on the basis of the morphological characters of their fruit bodies, the cultures exhibit no characters by which they may be assigned to genera or families. All identifications must go to the species directly. This means that keys to the species within a genus are of little aid in determining cultures from rots, and the use of this convenient generic grouping is denied the worker in this field. To meet an immediate need workers have prepared keys for the identification of the cultures isolated from a single host, for example, oak (Davidson, Campbell, and Vaughn (67) and Cartwright and Findlay (53)), black cherry (Davidson and Campbell (63)), balsam fir (Fritz (74)), and Sitka spruce (Baxter and Varner (26)); or on a group of host species, for example, softwoods in use in Great Britain (Cartwright and Findlay (54)) and hardwoods in Great Britain (Cartwright and Findlay (55)); or on a special habitat, for example, building timbers (Snell (138)) and railway ties (Walek-Czernecka (150)). In these keys, only slightly over 100 species are listed, which, in comparison with the number of species of wood-rotting fungi that may be encountered, is a small group. Consequently it may well happen that many fungi, when isolated and needing identification, cannot be found at all in any of the keys.

Furthermore, the diversity of methods adopted by the various authors and the different diagnostic characters used in their keys make it difficult to translate the data supplied by one author into terms that will permit comparison with data given by another author. Thus Fritz (74) grew cultures on potato dextrose slants at 22° C. in the dark; Cartwright and Findlay (53, 54, 55) used 2% malt agar slants in diffuse light at room temperature, and in the dark at temperatures of 20°, 25°, 30°, 35°, and 40° C., and on media containing gallic or tannic acids; Snell (138) used malt agar in Petri dishes at temperatures from 0° to 40° C.; and Baxter and Varner (26) grew cultures on 2% malt agar in Petri dishes at temperatures of 25°, 30°, and 35° C., and on wood blocks over a period of two years. For diagnostic characters, Baxter and Varner (26) relied on growth rates and macroscopic appearance of the cultures; Fritz (74) and Snell (138) used, in addition, microscopic characters; and Cartwright and Findlay (53, 54, 55) and Davidson, Campbell, and Vaughn (67) used, along with macroscopic and microscopic characters, the effect of the cultures on media containing gallic or tannic acid. Because of this lack of uniformity in methods and diagnostic characters, and the fact that the characters exhibited by the cultures are correlated with the conditions under which they are grown, it is necessary to follow each author's method in order to make use of his key. The difficulties to be met in identifying, for example, a culture from black cherry, one from balsam fir, and one from Sitka spruce, are immediately apparent.

The removal of these difficulties, at least in part, could be achieved by the adoption of a standard method acceptable to all those working on the cultural

characters of wood-rotting fungi, and the use of a key, such as that published by Davidson and his associates or the one used in the present paper, into which additional species, as they are described, could be incorporated. Under such a system it would be possible for each new worker to add to the information already accumulated, rather than, as so frequently happens now, to plan a new procedure and repeat studies on species whose cultural characters are known more or less completely from the work of other investigators.

Meanwhile the publication of an additional key has seemed advisable since it is more general in its application than those already available. It brings together under one system a large group of species, chosen without restriction as to host or locality, although all the species but one are known to occur in Canada. The key includes many of the species most frequently encountered in decay studies on various hosts and so may be considered a basic list. It is planned that, as additional species are examined in this Laboratory, their descriptions and key patterns will be published in conformity with the present publication. In this way it is hoped to establish a comprehensive key for the identification of cultures of the wood-decaying fungi likely to be encountered in Canada.

### Review of Literature

Much of the pertinent literature on cultures of wood-rotting fungi has been contributed by investigators who have studied cultures and sought means of identifying them for the practical purpose of determining the causes of decay in a given host or restricted habitat. Thus Cartwright and Findlay, from their work on the rots of English oak (53), hardwoods (55), and softwoods in use in Great Britain (54), contributed descriptions of the cultural characters of some 50 species of fungi. These descriptions are now available in the informative book, *Decay of Timbers and Its Prevention*, recently published by these authors (56). Davidson, Campbell, and Vaughn (67) described the cultures of 47 species causing decay in living oaks in the Eastern United States, and Davidson and Campbell (63) similarly described an additional nine species that cause decay in black cherry. Fritz (74) gave detailed descriptions of 18 species destructive to balsam fir and Snell (138), as a result of his investigations on the fungi causing decay in mill timbers, described five species in culture. Walek-Czernecka (150) published descriptions of the cultures of 21 species isolated from decays in railway ties in Poland, and Robak (134) gave comprehensive descriptions of six species important in the decay of coniferous wood in Norway.

Other investigators have studied the biology of a single species intensively and have published detailed information on its cultural characters, as, for example, Mounce (109) on *Fomes pinicola*, and Hilborn (83) on *F. fomentarius*. The variability encountered among several isolates of one species was the subject of studies by Hopp (87) and Verrall (149)—both of whom studied *Fomes igniarius*—and by Childs (57), working with *Polyporus Schweinitzii*, and Christensen (58), with *Polyporus circinatus*. Campbell (40) described the cultural characters of the species of *Fomes* and presented a key for their

identification. In his series of papers on resupinate polypores, Baxter (9 to 24) included cultural data for many species together with diagnostic keys based on cultural characters.

Much information on the physiology of wood-rotting fungi occurs incidentally in general accounts of various species, but several papers deal exclusively with physiologic reactions. Humphrey and Siggers (92) published data on the temperature relations of many species, and Snell, Hutchinson, and Newton (140) used temperature relations as a means of differentiating between the similar species, *Fomes roseus* and *Trameles subrosea*. Following a method originated by Bavendamm (5), Davidson, Campbell, and Blaisdell (64) tested the action on media containing tannic or gallic acid of about two hundred species of wood-inhabiting fungi. Of the 210 species, 36 produced no diffusion zone on either medium, 7 were inconsistent, while 166 produced diffusion zones. Of the 36 fungi that were consistently negative 30, or 83%, are associated with brown carbonizing rots; of 166 that were consistently positive 151, or 96%, are associated with white rots. Of the seven species giving inconsistent but mainly negative results six are definitely brown rot fungi while *Stereum subpileatum* is associated with a white pocket rot with darkening of the surrounding wood. They conclude "Thus Bavendamm's generalization, that brown rot fungi are negative when tested for oxidases by the use of gallic and tannic acids and that white rot fungi give positive reactions with the same media is essentially correct". Haas and Hill (78) state that the production of the brown diffusion zones is due to oxidation of the tannic or gallic acid. This reaction has proved to be of the utmost value as a diagnostic character.

The inhibitory action of certain contaminants on the growth of wood-decaying fungi in culture has been observed frequently. Overholts (123) recorded the antagonistic effect of a strain of *Penicillium notatum* on 29 species of fungi, including 22 that produce wood rots, and suggested the utilization of this phenomenon in identifying unknown cultures.

Most of the voluminous literature on interfertility phenomena in the Hymenomycetes is not relevant to a discussion of the identification of wood-rotting fungi, but one fact has emerged from it that has proved applicable. It has been demonstrated that, in heterothallic species, every monosporous mycelium obtained from one fruit body of a given species will pair with every monosporous mycelium from another fruit body of the same species from a different source so as to produce mycelium bearing clamp connections. This complete fertility is accepted as proof that the fruit bodies belong to the same species. By using this technique Mounce and Macrae (114) proved that the cultures derived from fruit bodies identified as *Fomes roseus* were interfertile, as were those isolated from fruit bodies identified as *F. subroseus*, but that no such interfertility occurred when pairings were made between isolates of *F. roseus* and *F. subroseus*. Thus additional evidence was obtained in support of the validity of the two species. Nobles (117) employed the same method to show that numerous cultures isolated from decays in coniferous wood were identical with a culture derived from a fruit body, described as *Poria microspora*

Overholts, but were distinct from cultures of *Trametes serialis*, which had formerly been considered the cause of the decay. This technique has practical applications in identification of cultures, since it may be applied to corroborate the identification of isolates that fruit in culture and from which monosporous cultures can be obtained.

Additional references that contain information on the cultural characters of individual species are cited under the appropriate species. The considerable volume of literature, especially that of recent publication, on the cultural characters of wood-decaying fungi and the identification of these fungi on the basis of their cultural characters, indicates the importance of this work.

### Cultures

The cultures in the collection of wood-inhabiting fungi in the Division of Botany and Plant Pathology, Department of Agriculture, Ottawa, were the basis of the descriptions given in the present paper. This collection, established in 1924, was enlarged during the succeeding years by Dr. Irene Mounce and her associates. By the end of 1946 it contained 1265 named cultures, representing 340 species in 62 genera. In general, the aim has been to insure the authenticity of the cultures by isolating them from fruit bodies properly identified and deposited in the Mycological Herbarium, Department of Agriculture, Ottawa. However, some isolates received from contributors lack accompanying specimens, and others were obtained from rots not associated with fruit bodies. Most species are represented by a number of isolates, preferably from different hosts and geographic localities. It has been the practice to obtain cultures from the United States, Europe, and Asia, whenever possible, so that for many species cultures from widely separated localities are available for comparison. For a number of species there are, in addition, isolates from both fruit bodies and associated rots. The collection includes, therefore, not only cultures of a large number of species but also a sufficient number of isolates within the species to permit study and comparison. With few exceptions the cultures have remained in good condition, early isolates showing little deterioration during the long period they have been in culture.

### Methods

To facilitate its use by other workers, the descriptions and key have been based on cultural characters, both macroscopic and microscopic, that can be demonstrated readily without the aid of elaborate equipment or methods. The descriptions include only those characters shown by the cultures when grown on malt agar and malt agar containing gallic or tannic acids, prepared according to the following formulae.

#### MALT AGAR

Difco powdered malt.....	12.5 gm.
Difco Bacto-agar.....	20.0 gm.
Distilled water.....	1000.0 cc.



The malt is added to the agar dissolved in water, filtered through several layers of cheesecloth, and sterilized for 20 min. at 15 lb. pressure.

#### GALLIC (OR TANNIC) ACID AGAR

Difco powdered malt.....	15.0 gm.
Difco Bacto-agar.....	20.0 gm.
Distilled water.....	1000.0 cc.
Gallic (or tannic) acid.....	5.0 gm.

This medium is prepared according to the method described by Davidson, Campbell, and Blaisdell (64), the agar and malt being added to 850 cc. of water, the remaining 150 cc. of water being placed in a separate flask. The contents of both flasks are sterilized for 20 min. at 15 lb. pressure. While the sterilized water is still hot, the gallic (or tannic) acid is dissolved in it, and this solution is added to the slightly cooled malt agar and thoroughly mixed with it before being poured directly into sterile Petri dishes.

Each isolate to be studied is grown on malt agar in a Petri dish for one week. From this actively growing culture a cube, two to three mm. square, is transferred to the edge of each of five 10 cm. Petri dishes containing about 30 cc. of malt agar. The cultures are incubated in the dark at room temperature, being brought into the light only for examination. They are examined and described at weekly intervals for six weeks, one being removed for photographing at the end of two weeks, four weeks, and six weeks. The Petri dishes containing gallic and tannic acid agars are inoculated in the center with cubes four to five mm. square, taken from four- to six-weeks-old cultures. The cultures on gallic or tannic acid media are examined and discarded at the end of one week, except in those cases where the effect on the medium is weak or difficult to determine.

The records for each isolate include data on the rate of growth, form, and character of the advancing zone, color and topography of the mat, the presence or absence of fruit bodies, the effect of the growth of the fungus on the color of the agar ("Reverse"), the odor, and the reaction on media containing gallic and tannic acids. In addition, the records contain descriptions, accompanied by *camera lucida* drawings, of the microscopic characters of the isolate based on the examination at suitable intervals of preparations from the advancing zone, aerial mycelium, and submerged mycelium. Preparations for microscopic examination are mounted in a 7% aqueous solution of potassium hydroxide and stained in an aqueous solution of phloxine.

In the hope that the description may allow for all or most of the variations that may be observed in cultures of the species and at the same time exclude other species, an attempt has been made to make the description cover the range of characters encountered in the several isolates, rather than to describe one typical isolate. The cultures on which the description for each species is based are listed under their numbers in the Mycological Herbarium, Department of Agriculture, Ottawa, along with host and locality in which the collection was made.

In the descriptions, the rate of growth is given in terms of the number of weeks required by the fungus to cover the agar in the Petri dish from the inoculum at the periphery of the dish. Those species that grow so slowly that the agar is not covered in six weeks are treated in one group under rate of growth. It appears that this system of describing growth rates will give sufficient range to include the variations in rate of growth that may be encountered when many isolates are grown at laboratory temperatures, which may vary considerably from time to time.

Throughout the study colors have been described according to Ridgway's (131) *Color Standards and Color Nomenclature*, but it is proposed to adopt the *Munsell Book of Color* (116) for future work. In the descriptions, the Munsell color equivalents are given in parentheses following the Ridgway names. These Munsell notations are considered to represent reasonably accurately the chips in the volume of Ridgway used. Complete accuracy of correlation with chips in other copies of Ridgway is impossible since the original colors are subject to change and cannot be reproduced satisfactorily. Free use has been made of Dr. D. H. Hamly's list of equivalents in the two systems, additional equivalents have been supplied by Mrs. B. R. Bellamy of the Munsell Color Company, Baltimore, and all notations have been checked by Dr. Hamly and Mr. W. I. Illman at the Department of Botany, University of Toronto. If the *Munsell Book of Color* is to replace Ridgway's *Color Standards and Color Nomenclature* in describing colors in the biological sciences, it would appear helpful to include both systems in descriptions during the transition period.

In describing the topography and texture of the mats, the terms used are largely those first employed by Long and Harsch (98), together with some introduced by Campbell (42). These terms, with their authors' original definitions, are included in the list below, the letters "L. and H." indicating that the definition was taken from Long and Harsch's paper and the letter "C.", from Campbell's paper. Microscopic structures in the cultures are usually identical with or similar to those present in the fruit bodies. Consequently the same descriptive terms are applicable to both. An attempt has been made to define these terms in the sense in which they are used in the present paper, but the definitions will probably apply equally well in broader usage.

#### TERMS USED IN DESCRIBING ADVANCING ZONE:

*Even*.—Margin a smooth line, without indentations.

*Bayed*.—Margin indented, scalloped.

*Submerged*.—Mycelium growing through the agar, below its surface.

*Appressed*.—"Mycelium which is prostrate on the surface of the agar. This with many fungi is the first stage in the aerial growth of the mycelium. Later this appressed mycelium may give place to other forms" (L. and H.).

*Raised*.—"Forming a mound on agar as opposed to appressed" (C.).

## TERMS USED IN DESCRIBING TEXTURE OF MAT:

*Chamoislike*.—Smooth, with the texture of fine chamois.

*Cottony*.—"Erect, rather long (3 to 5 mm.) mycelium spreading in all directions" (L. and H.).

*Crustose*.—Forming a crust, usually dark in color.

*Downy*.—"Short, fine hairs, loosely scattered over the surface of the mycelium, giving it a downy appearance" (L. and H.).

*Farinaceous*.—Having a mealy surface, powdery.

*Felty*.—"Matted with intertwined hairs, resembling felt" (L. and H.).

*Floccose*.—"Scattered patches of short mycelium" (L. and H.).

*Lacunose*.—Covered with pits or depressions.

*Plumose*.—"Tufts of mycelium with a central axis from which short hyphae radiate" (L. and H.).

*Plushlike*.—Similar to velvety, but with a thicker 'pile'.

*Silky*.—"Long parallel threads of mycelium, more or less prostrate, like combed silk" (L. and H.).

*Subfelty*.—"A thin layer of mycelium consisting of short intertwined hairs" (L. and H.).

*Velvety*.—"Layer of mycelium with distinct, dense, straight, short hairs like pile of velvet" (L. and H.).

*Woolly*.—"A dense mass of mycelium consisting of long, tortuous, matted hairs. Cottony and woolly may both later become felty by the long hairs becoming matted and prostrate" (L. and H.).

## TERMS USED IN DESCRIBING MICROSCOPIC CHARACTERS:

*Bulbil*.—Small compact mass of hyphae, apparently formed by one or more branches coiling tightly around a parent hypha, as in *Fomes nigrolimitatus*. Listed in the key as "3" under special structures (Pl. IV, Fig. 29).

*Contorted incrusted hyphae*.—Hyphal ends, usually with short irregular branches, covered with crystalline material, as in *Polyporus albellus* (Pl. VIII, Fig. 20) and *P. semipileatus* (Pl. XIV, Fig. 7). Listed in the key as "0" under special structures.

*Cuticular cells*.—Swollen cells, at first hyaline, with contents staining in phloxine, then empty, the walls remaining colorless, as in *Ganoderma applanatum* (Pl. VI, Fig. 4), or becoming brown, as in *Fomes igniarius* (Pl. IV, Fig. 19). Listed in the key as "5" under special structures.

*Cystidia*.—Hyaline structures, produced on aerial mycelium, resembling the sterile organs occurring with the basidia in the hymenium of many species of Hymenomycetes, seen in cultures of *Polyporus abietinus* (Pl. VIII, Fig. 14), *Echinodontium tinctorium* (Pl. II, Fig. 30), and others. Listed in the key as "1" under special structures.

*Dendritic*.—Treelike branching exhibited by some hyphae in *Polyporus anceps* (Pl. VIII, Fig. 26).

*Fiber hyphae*.—Hyphae with thick refractive walls, hyaline or brown, and lumina narrow or apparently lacking, observed in many species, as for example, *Ganoderma applanatum* (Pl. VI, Fig. 2) and *Lenzites trabea* (Pl. VI, Fig. 45).

*Helicoid hyphae*.—Hyphae with ends in a coiled or spiral form, as in *Fomes fulvus* (Pl. IV, Fig. 14).

*Hyphae with numerous interlocking projections*.—Hyphae forming a pseudo-parenchymatous layer, as seen in cultures of *Polyporus dryophilus* var. *vulpinus* (Pl. X, Fig. 33). Listed in the key as "6" under special structures.

*Lactiferous cells*.—Large cells, up to  $30\mu$  in diameter, with deeply staining contents, conspicuous in mounts from *Polyporus resinusus* (Pl. XII, Fig. 49). Listed in the key as "8" under special structures.

*Nodose-septate*.—With clamp connections at the septa.

*Secondary spores*.—Spores produced by species of Hymenomycetes, but not borne on basidia,—

*Chlamydospores*, secondary spores with walls more or less thickened, hyaline or colored, borne at the end of (terminal) or within (intercalary) ordinary vegetative hyphae, as in *Daedalea quercina* (Pl. II, Fig. 21).

*Conidia*, secondary spores with thin walls, borne terminally on specialized structures, the conidiophores, as in *Fomes annosus* (Pl. II, Fig. 34), and *Polyporus sulphureus* (Pl. XIV, Figs. 13, 14).

*Oidia*, secondary spores with thin walls, produced by the fragmentation of ordinary vegetative hyphae, as in *Collybia velutipes* (Pl. II, Fig. 8).

*Rigid hyphae*.—Hyphae with right-angled branching and refractive walls, conspicuous in mounts from *Poria carbonica* (Pl. XVI, Fig. 9). Listed in the key as "4" under special structures.

*Setae*.—Yellow or brown, thick-walled structures, borne on aerial mycelium, resembling the setae present in the fruit bodies of many species of Hymenomycetes, seen in cultures of *Fomes Pini* (Pl. IV, Fig. 38), *Polyporus gilvus* (Pl. XII, Fig. 5), and others. Listed in the key as "2" under special structures.

*Setal hyphae*.—Dark brown, thick-walled hyphae with setalike ends, occurring in *Poria Weirii* (Pl. XVI, Fig. 45) and in other species. Listed in the key as "2" under special structures.

*Staghorn hyphae*.—Hyphae with clumps of repeatedly branched hyphae, the branching usually dichotomous, observed in several species of *Ganoderma* (Pl. VI, Figs. 3, 7, 11, and 19) and elsewhere.

In the use of media containing gallic and tannic acids, the methods of studying and recording the reactions have been those of Davidson, Campbell, and Blaisdell (64), the following terms descriptive of the strength of the reactions being substituted for the symbols used by these authors.

*Lacking*.—"—, Negative, no brown discoloration of the agar under or about the mat."

*Very weak.*—" +, Diffusion zone light to dark brown, formed under inoculum at center of mat and visible only from under side of dish. In case no growth takes place, a faint brown discoloration under the inoculum."

*Weak.*—" ++, Diffusion zone light to dark brown, formed under most of mat but not extending to margin. Visible from under side only."

*Moderately strong.*—" +++, Diffusion zone light to dark brown, extending a short distance beyond the margin of the mat and visible from the upper side."

*Strong.*—" ++++, Diffusion zone dark brown, opaque, extending considerably beyond margin of fungus mat."

*Very strong.*—" +++++, Diffusion zone very intense, dark brown, opaque, forming a wide corona about mat. Usually such intense reactions occur with species giving no growth on the medium, and are most common on gallic acid medium."

Accompanying the description of each species are brief notes on the type of rot produced by the species, and its host range. This information has been obtained from the literature, especially from Boyce (35), Lowe (99, 100), Overholts (119, 120), and Shope (136), as well as from records in the Department of Agriculture, Ottawa, Mycological Herbarium, and personal observation. The lists of references to publications containing data on the cultural characters of the species described are not claimed to be complete, but they indicate the literature that has been readily available.

### Key for the Identification of Cultures of Wood-rotting Fungi

The key provides for the identification of the cultures of 126 species of wood-rotting fungi, the majority of which belong to the Polyporaceae, with fewer representatives from the Agaricaceae, Thelephoraceae, and Hydnaceae. The list includes many of the species commonly encountered in decay studies on various hosts and may be considered as a basic list.

As will be observed, the key consists of a series of "key patterns" (Davidson, Campbell, and Vaughn (67)) arranged in ascending numerical order. Each column of digits in the key refers to a specific diagnostic character. The first digit refers to the host, the second to the color of the mycelial mat, the third to the reaction on media containing gallic and tannic acids, and so on. The actual digit in each column has a precise meaning. Thus, in the first column, "1" indicates that the host is a broad-leaved tree, "2" that it is a coniferous tree; in the second column, "1" indicates that the mycelial mat remains white or pale for six weeks, "2" that it becomes yellow or brown, at least when mature; in the fourth column, "1" means that clamp connections are regularly present in all parts of the mat, "2" that all hyphae have simple septa, "3" that hyphae of the advancing zone have simple septa while those of the older part of the mat have clamp connections, "4" that multiple clamp connections are present.

The following list indicates the meaning attached to each digit as it is used in each of the columns.

First column.—Host.

1. Occurring on broad-leaved trees.
2. Occurring on coniferous trees.

Second column.—Color of mycelial mat.

1. Mat remaining white or pale yellow or pale pink for six weeks.
2. Mat yellow or brown, at least when mature.

Third column.—Reaction on media containing gallic and tannic acid agars.

1. Diffusion zone present.
2. Diffusion zones lacking.

Fourth column.—Septation of hyphae.

1. Clamp connections regularly present on all parts of mat, although fiber hyphae lacking them may occur.
2. Simple septa on all hyphae.
3. Hyphae of advancing zone with simple septa, those of older part of mat with clamp connections.
4. Multiple clamp connections present, at least in the advancing zone.

Fifth column.—Special structures.

- 0 to 8. Special structures present.
  0. Contorted incrustated hyphae.
    1. Cystidia or gloecystidia.
    2. Setae or setal hyphae.
    3. Bulbils.
    4. Rigid hyphae with right angled branches.
    5. Cuticular cells, forming pseudoparenchymatous layer.
    6. Hyphae with numerous interlocking projections.
    7. Swellings on hyphae.
    8. Lactiferous cells.
  9. No special structures.

Sixth column.—Chlamydospores.

1. Chlamydospores present.
2. Chlamydospores lacking.

Seventh column.—Conidia.

1. Conidia present.
2. Conidia lacking.

Eighth column.—Oidia.

1. Oidia present.
2. Oidia lacking.

Ninth column.—Rate of growth.

1. Rate of growth rapid, plates covered in one to two weeks.
2. Rate of growth moderately rapid, plates covered in three to four weeks.
3. Rate of growth slow, plates covered in five to six weeks.
4. Rate of growth very slow, plates not covered in six weeks.

Tenth column.—Fruiting.

1. Fruiting before the end of six weeks.
2. No fruiting.

Eleventh column.—Effect on agar.

1. Reverse brown, at least in part, before the end of six weeks.
2. Reverse unchanged, or not darker than honey yellow in six weeks.
3. Reverse bleached, at least in part, before the end of six weeks.

For a given species the key pattern is prepared by considering the diagnostic characters of the species in the order in which they occur in the key and choosing the proper digit for each column. Thus *Polyporus anceps* occurs on coniferous trees (2); its mat remains white (1); it has positive reactions on media containing gallic and tannic acid (1); it has clamp connections (1), no special structures (9), no chlamydospores (2), no conidia (2), no oidia (2); its growth rate is rapid (1); it fruits (1); and the reverse remains unchanged (2). The key pattern is, therefore,

2 1 1 1 9 2 2 2 1 1 2 .

Under the method employed, most of the species vary in one or more characters. For example, *Fomes pinicola* may occur on both broad-leaved and coniferous trees (1,2), may or may not produce chlamydospores (1,2), and may grow moderately rapidly or slowly (2,3), and its key pattern, to show these possible variations must be

(1,2) 1 2 1 9 (1,2) 2 2 (2,3) 2 2 .

In the key, those species that do not vary in any of the diagnostic characters occur only once, those that have one variable character occur twice, those with two variable characters four times, and those with three variable characters, eight times, since provision must be made for all possible combinations of the variable characters. When the key patterns for all the species are arranged in numerical order, it becomes apparent that several groups of species have identical key patterns. Within such groups, separation must be made on the basis of characters impossible to include in a numerical key, such as texture and topography of mat, color, host specificity, and so on. Consequently it has been necessary to insert descriptive keys. Frequently the notes accompanying the individual descriptions will aid in separating two species having identical key patterns, and in such cases descriptive keys have been omitted.

When an unknown culture is being identified, its cultural characters must be determined under the conditions prescribed, and its key patterns prepared and matched with those in the key. Comparison must then be made with the descriptions of species with identical key patterns, and where possible, with living cultures.

## KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	1	1	1	0	1	2	2	2	1	2	----- <i>Polyporus albellus</i>
1	1	1	1	0	1	2	2	2	2	2	----- <i>Polyporus albellus</i>
1	1	1	1	0	2	2	2	4	2	1	----- <i>Polyporus semipileatus</i>
1	1	1	1	0	2	2	2	4	2	2	----- <i>Polyporus semipileatus</i>
1	1	1	1	1	2	2	2	2	1	2	----- <i>Polyporus pargamensis</i>
1	1	1	1	1	2	2	2	2	2	2	----- <i>Polyporus abietinus</i>
											----- <i>Polyporus pargamensis</i>
1	1	1	1	5	1	2	2	1	2	3	----- <i>Ganoderma lucidum</i>
1	1	1	1	5	1	2	2	2	1	2	----- <i>Fomes fraxineus</i>
1	1	1	1	5	1	2	2	2	2	2	----- <i>Fomes fraxineus</i>
1	1	1	1	5	2	2	1	2	2	1	----- <i>Polyporus graveolens</i>
1	1	1	1	5	2	2	1	2	2	2	----- <i>Polyporus graveolens</i>
1	1	1	1	5	2	2	2	2	2	2	----- <i>Ganoderma applanatum</i>
1	1	1	1	5	2	2	2	4	2	2	----- <i>Ganoderma applanatum</i>
1	1	1	1	6	2	2	1	4	2	2	----- <i>Polyporus squamosus</i>
1	1	1	1	6	2	2	2	2	2	2	----- <i>Collybia radicata</i>
1	1	1	1	9	1	2	2	1	1	2	Chlamydospores rare; basidiospores cylindric, 5.4-7.5 × 2.2-2.7μ----- <i>Polyporus hirsutus</i>
											Chlamydospores numerous; basidiospores ovoid, trun- cate, 6.0-9.0 × 4.5-6.0μ; always on <i>Quercus</i> spp. ----- <i>Polyporus compactus</i>
											Chlamydospores numerous; basidiospores rare, broadly ovoid, 5.4 × 3.6μ----- <i>Polyporus distortus</i>
1	1	1	1	9	1	2	2	1	2	2	----- <i>Polyporus hirsutus</i>
											----- <i>Polyporus zonatus</i>
1	1	1	1	9	1	2	2	1	2	3	Chlamydospores numerous, 7.5-15.0 × 4.5-6.0μ; odor very sweet----- <i>Trameles suaveolens</i>
											Chlamydospores rare to numerous, 6.0-12.0 × 4.5-7.5μ; no odor----- <i>Polyporus zonatus</i>
											Chlamydospores rare to numerous, 4.5-7.5 × 2.2- 4.5μ; strong 'fishy' odor----- <i>Polyporus versicolor</i>
1	1	1	1	9	1	2	2	2	1	1	----- <i>Polyporus frondosus</i>
1	1	1	1	9	1	2	2	2	1	2	Mat felty, 'corroded', fruiting in compact pored areas; chlamydospores numerous, with thick sculptured walls, 10.5-16.5 × 7.5-12.0μ; basidiospores sub- globose or broadly ovoid, 4.5-6.0μ ----- <i>Fomes fraxineus</i>
											Mat felty to chamoislike, frequently fruiting along radii; chlamydospores thin-walled, 9.0-18.0 × 6.0-9.0μ; basidiospores ovoid, 6.0-7.5 × 4.5-6.0μ ----- <i>Fomes fraxinophilus</i>
											Mat cottony-woolly, with scattered balls of soft cottony mycelium, later waxy and pored; chlamydo- spores numerous, thin-walled, 6.0-12.0 × 4.5- 7.0μ; basidiospores hyaline, allantoid, 3.0-4.0 × 1.5μ----- <i>Polyporus albellus</i>
											Mat felty to chamoislike, with overgrowth of tufted mycelium, where fruiting occurs; chlamydospores numerous, thick-walled, 8.0-13.5 × 6.0-9.0μ; basi- diospores ovoid, truncate, 6.0-9.0 × 4.5-6.0μ ----- <i>Polyporus compactus</i>



# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—*Continued*

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	1	1	1	9	1	2	2	2	1	2	Mat pink, downy to cottony-floccose; chlamydospores rare, thin-walled, $10.5-24.0 \times 6.0-7.5\mu$ ; basidiospores cylindric, curved, $6.0-8.0 \times 1.5-2.0\mu$ ----- <i>Fomes subroseus</i>
											Mat woolly-felty, fruiting in compact areas; strong odor of carbide; chlamydospores few to abundant, walls slightly thickened, $10.5-22.0 \times 9.0-16.5\mu$ ; basidiospores ellipsoid to ovoid, $5.0-6.5 \times 3.5-4.5\mu$ ----- <i>Polyporus frondosus</i>
											Mat cottony, translucent except for dots or larger areas opaque, subfelty to waxy, minutely pored; chlamydospores numerous, with walls somewhat thickened, $7.5-13.5 \times 4.5-9.0\mu$ ; basidiospores ellipsoid to oblong, $3.6-4.5 \times 2.7\mu$ ----- <i>Polyporus fumosus</i>
											Mat woolly-felty to plasterlike, with large compact lumps that may become pored; chlamydospores rare or apparently lacking, thin-walled, $10.5-16.5 \times 4.5-7.0\mu$ ; basidiospores cylindric, $5.4-7.2 \times 2.2-2.7\mu$ ----- <i>Polyporus hirsutus</i>
											Mat raised, woolly, with typical fruit bodies on surface, distorted ones growing between halves of Petri dish; chlamydospores with walls slightly thickened, $6.0-15.0 \times 4.5-7.5\mu$ ; basidiospores cylindric, $3.0-4.0 \times 1.5\mu$ ----- <i>Schizophyllum commune</i>
1	1	1	1	9	1	2	2	2	2	1	----- <i>Polyporus frondosus</i>
1	1	1	1	9	1	2	2	2	2	2	Mat felty, 'corroded'; chlamydospores numerous, with thick sculptured walls, $10.5-16.5 \times 7.5-12.0\mu$ ----- <i>Fomes fraxineus</i>
											Mat fine woolly, with few scattered compact lumps; chlamydospores numerous, thin-walled, $7.5-12.0 \times 6.0-9.0\mu$ ----- <i>Lentinus tigrinus</i>
											Mat cottony-woolly with scattered balls of soft cottony mycelium; chlamydospores numerous, thin-walled, $6.0-12.0 \times 4.5-7.0\mu$ ----- <i>Polyporus albellus</i>
											Mat woolly-felty; chlamydospores few to abundant, walls slightly thickened, $10.5-22.0 \times 9.0-16.5\mu$ ----- <i>Polyporus frondosus</i>
											Mat cottony, translucent except for scattered dots or larger areas that are opaque and subfelty; chlamydospores numerous, with walls somewhat thickened, $7.5-13.5 \times 4.5-9.0\mu$ ----- <i>Polyporus fumosus</i>
											Mat woolly-felty to plasterlike, with large compact lumps; chlamydospores rare or apparently lacking, thin-walled, $10.5-16.5 \times 4.5-7.0\mu$ ----- <i>Polyporus hirsutus</i>
											Mat raised, woolly; chlamydospores with walls slightly thickened, $6.0-15.0 \times 4.5-7.5\mu$ ----- <i>Schizophyllum commune</i>
1	1	1	1	9	1	2	2	2	2	3	----- <i>Polyporus versicolor</i> ----- <i>Trametes suaveolens</i>
1	1	1	1	9	1	2	2	3	1	1	----- <i>Polyporus frondosus</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—*Continued*

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	1	1	1	9	1	2	2	3	1	2	----- <i>Polyporus frondosus</i> <i>Polyporus obtusus</i>
1	1	1	1	9	1	2	2	3	2	1	----- <i>Polyporus frondosus</i>
1	1	1	1	9	1	2	2	3	2	2	Chlamydospores few to abundant, with walls slightly thickened, $10.5-22.0 \times 9.0-16.5\mu$ ; strong odor of carbide----- <i>Polyporus frondosus</i> Chlamydospores numerous, thin-walled, $6.0-13.5 \times 6.0-9.0\mu$ ; odor strong, fragrant----- <i>Polyporus obtusus</i> Chlamydospores numerous, usually rough-walled, $7.5-21.0 \times 4.5-12.0\mu$ ; odor of iodoform strong----- <i>Poria asiatica</i>
1	1	1	1	9	1	2	2	4	2	2	----- <i>Poria asiatica</i>
1	1	1	1	9	2	1	2	2	2	2	----- <i>Pholiota adiposa</i>
1	1	1	1	9	2	1	2	3	2	2	----- <i>Pholiota adiposa</i>
1	1	1	1	9	2	2	1	4	2	1	----- <i>Polyporus umbellatus</i>
1	1	1	1	9	2	2	1	4	2	2	----- <i>Polyporus squamosus</i> <i>Polyporus umbellatus</i>
1	1	1	1	9	2	2	2	1	1	2	----- <i>Polyporus hirsutus</i> <i>Polyporus pubescens</i>
1	1	1	1	9	2	2	2	1	1	3	----- <i>Polyporus pubescens</i>
1	1	1	1	9	2	2	2	1	2	2	Mat at first floccose-woolly, becoming patchy, with some areas raised, felty-woolly, intervening areas appressed, thin felty----- <i>Lenzites betulina</i> Mat woolly-felty to plasterlike, with large compact lumps----- <i>Polyporus hirsutus</i> Mat cottony at first, then uniformly felty, more or less pitted----- <i>Polyporus pubescens</i> Mat uniformly cottony-woolly to felty, frequently with minute papillae where mat is grown against side of Petri dish----- <i>Poria subacida</i>
1	1	1	1	9	2	2	2	1	2	3	Mat at first woolly-floccose, becoming appressed, woolly-felty, with slightly raised reticulations----- <i>Daedalea unicolor</i> Mat appressed, subfelty to floccose to somewhat pitted, except in part remote from inoculum where it is raised, woolly----- <i>Polyporus conchifer</i> Mat cottony at first, then uniformly felty, more or less pitted----- <i>Polyporus pubescens</i> Mat raised, cottony-woolly in newer growth and remaining so in some areas, otherwise felty, with drops of exudate that may leave surface punctate----- <i>Polyporus versicolor</i> Mat uniformly cottony-woolly to felty, frequently with minute papillae grown against side of Petri dish----- <i>Poria subacida</i>
1	1	1	1	9	2	2	2	2	1	1	----- <i>Fomes roseus</i>
1	1	1	1	9	2	2	2	2	1	2	Mat pale pink, fruiting surface diffuse, granular to irregularly pored----- <i>Fomes roseus</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—*Continued*

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	1	1	1	9	2	2	2	2	1	2	Mat white, fruit bodies distorted mushrooms with gills ----- <i>Pleurotus ostreatus</i>
											Mat white, pored fruiting surfaces formed on large lumps over inoculum and elsewhere ----- <i>Polyporus hirsutus</i>
											Mat white, fruit bodies with gills, normal or distorted ----- <i>Schizophyllum commune</i>
1	1	1	1	9	2	2	2	2	2	2	Mat usually becoming buffy brown to olive brown in small areas and in lines traversing white mat, thong- like strands grown through agar conspicuous from lower side----- <i>Collybia radicata</i>
											Mat white, appressed downy; occurring only on <i>Shepherdia argentea</i> ----- <i>Fomes Ellisianus</i>
											Mat at first floccose-woolly, becoming patchy, with some areas raised, felty-woolly, intervening areas appressed, thin felty----- <i>Lenzites betulina</i>
											Mat white, appressed, woolly-felty, with slightly raised tufts arranged in more or less concentric lines, producing zonate appearance ----- <i>Pleurotus ostreatus</i>
											Mat woolly-felty to plasterlike, with large compact lumps----- <i>Polyporus hirsutus</i>
											Mat uniformly cottony-woolly to felty, frequently with minute papillae grown against side of Petri dish ----- <i>Poria subacida</i>
											Mat raised woolly, with scattered, more or less compact lumps----- <i>Schizophyllum commune</i>
1	1	1	1	9	2	2	2	2	2	3	Mat appressed, subfelty to floccose to somewhat pitted, except in part remote from inoculum where it is raised, woolly----- <i>Polyporus conchifer</i>
											Mat raised, cottony-woolly in newer growth and remaining so in some areas, otherwise felty, with drops of exudate that may leave surface punctate ----- <i>Polyporus versicolor</i>
											Mat uniformly cottony-woolly to felty, frequently with minute papillae grown against side of Petri dish----- <i>Poria subacida</i>
1	1	1	1	9	2	2	2	3	2	2	----- <i>Favolus alveolaris</i>
											----- <i>Fomes Ellisianus</i>
1	1	1	1	9	2	2	2	3	2	3	----- <i>Favolus alveolaris</i>
1	1	1	1	9	2	2	2	4	2	2	----- <i>Fomes ohiensis</i>
											----- <i>Stereum Murraii</i>
1	1	1	2	7	2	2	2	4	2	2	----- <i>Fomes connatus</i>
											----- <i>Trametes tenuis</i>
1	1	1	2	9	1	1	2	3	2	2	----- <i>Polyporus Berkeleyi</i>
1	1	1	2	9	1	2	2	3	1	2	----- <i>Polyporus Berkeleyi</i>
1	1	1	2	9	2	1	2	1	2	2	----- <i>Fomes annosus</i>
1	1	1	2	9	2	1	2	4	2	2	----- <i>Fomes annosus</i>
1	1	1	2	9	2	2	2	1	2	1	----- <i>Hymenochaete corrugata</i>
1	1	1	2	9	2	2	2	1	2	2	----- <i>Hymenochaete corrugata</i>

KEY FOR THE IDENTIFICATION OF CULTURES OF  
WOOD-ROTTING FUNGI—Continued

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	1	1	2	9	2	2	2	1	2	3	----- <i>Polyporus Tulipiferae</i>
1	1	1	2	9	2	2	2	2	2	1	----- <i>Hymenochaete corrugata</i>
											----- <i>Polyporus dryophilus</i>
1	1	1	2	9	2	2	2	2	2	2	----- <i>Hymenochaete corrugata</i>
1	1	1	2	9	2	2	2	2	2	3	----- <i>Polyporus dryophilus</i>
											----- <i>Polyporus Tulipiferae</i>
1	1	1	2	9	2	2	2	3	2	1	----- <i>Polyporus dryophilus</i>
1	1	1	2	9	2	2	2	3	2	2	----- <i>Poria ferrea</i>
1	1	1	2	9	2	2	2	3	2	3	----- <i>Polyporus dryophilus</i>
											----- <i>Poria ferrea</i>
1	1	1	2	9	2	2	2	4	2	2	----- <i>Fomes connatus</i>
											----- <i>Trameles tenuis</i>
1	1	1	3	8	1	2	2	1	2	2	----- <i>Polyporus resinosus</i>
1	1	1	3	8	1	2	2	1	2	3	----- <i>Polyporus resinosus</i>
1	1	1	3	8	2	2	2	1	2	2	----- <i>Polyporus resinosus</i>
1	1	1	3	8	2	2	2	1	2	3	----- <i>Polyporus resinosus</i>
1	1	1	3	9	1	2	1	1	2	2	----- <i>Poria albipellucida</i>
											----- <i>Poria cinerescens</i>
1	1	1	3	9	1	2	1	1	2	3	----- <i>Poria albipellucida</i>
											----- <i>Poria cinerescens</i>
1	1	1	3	9	1	2	2	1	2	2	----- <i>Polyporus resinosus</i>
											----- <i>Poria albipellucida</i>
											----- <i>Poria cinerescens</i>
1	1	1	3	9	1	2	2	1	2	3	Mat at first all downy, later with woolly areas, appearing patchy; chlamydospores numerous, globose to ovoid, 6.0–12.0 $\mu$ diameter; no odor
											----- <i>Polyporus galactinus</i>
											Mat at first thin woolly and subfelty, later with raised cottony areas, appearing patchy; chlamydospores numerous to rare, 16.0–24.0 $\times$ 9.0–16.5 $\mu$ ; odor strong, sweet.----- <i>Polyporus resinosus</i>
											Mat patchy, with conspicuous V-shaped thickenings, opaque because of denser growth within agar and fine woolly to farinaceous surface; chlamydospores usually numerous, 7.5–10.5 $\mu$ diameter; odor of apples.----- <i>Poria albipellucida</i>
											----- <i>Poria cinerescens</i>
1	1	1	3	9	2	2	2	1	2	2	----- <i>Polyporus resinosus</i>
1	1	1	3	9	2	2	2	1	2	3	----- <i>Polyporus resinosus</i>
1	1	2	1	9	1	2	2	1	1	2	----- <i>Poria monticola</i>
1	1	2	1	9	1	2	2	1	2	2	----- <i>Poria monticola</i>
1	1	2	1	9	1	2	2	2	1	2	Mat white, woolly-felty, with slightly raised reticulations, fruiting over raised tufts; chlamydospores numerous, thick-walled, 12.0–18.0 $\times$ 7.5–13.5 $\mu$ ; basidiospores oblong-ellipsoid to cylindric, 6.0–8.0 $\times$ 2.5–3.0 $\mu$ .----- <i>Daedalea quercina</i>
											Mat pink, downy to floccose, fruiting over most of surface; chlamydospores rare, thin-walled, 10.5–24.0 $\times$ 6.0–7.5 $\mu$ ; basidiospores cylindric, curved, 6.0–8.0 $\times$ 1.5–2.0 $\mu$ .----- <i>Fomes subroseus</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—*Continued*

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	1	2	1	9	1	2	2	2	1	2	Mat white, cottony, translucent except for opaque dots or larger areas, subfely to waxy, minutely pored; chlamydospores numerous, with walls somewhat thickened, $7.5-13.5 \times 4.5-9.0\mu$ ; basidiospores ellipsoid to oblong, $3.6-4.5 \times 2.7\mu$ ----- <i>Polyporus fumosus</i>
											Mat white, raised woolly, with typical or distorted fruit bodies; chlamydospores with walls slightly thickened, $6.0-15.0 \times 4.5-7.5\mu$ ; basidiospores cylindric, $3.0-4.0 \times 1.5\mu$ . <i>Schizophyllum commune</i>
											Mat white or with tinge of pale "cartridge buff" to "cinnamon-buff", cottony to woolly, forming foliose fruit bodies; chlamydospores numerous to rare, with walls slightly thickened, $8.0-19.5 \times 6.0-13.5\mu$ ; basidiospores oblong-ellipsoid, $4.5-6.0 \times 2.2-2.7\mu$ ----- <i>Poria monticola</i>
											Mat white, newest growth raised, cottony, then collapsed, leaving thin film on agar; chlamydospores rare or apparently lacking, $10.5-19.5 \times 6.0-7.5\mu$ ; basidiospores cylindric, slightly curved, $4.2-5.0 \times 1.2\mu$ ----- <i>Poria xantha</i>
1	1	2	1	9	1	2	2	2	2	2	Mat white, cottony-woolly; chlamydospores numerous in some isolates, usually limited to advancing zone, $6.0-18.0 \times 6.0-9.0\mu$ ----- <i>Fomes pinicola</i> (See immediately above) <i>Daedalea quercina</i> <i>Polyporus fumosus</i> <i>Poria monticola</i> <i>Poria xantha</i> <i>Schizophyllum commune</i>
1	1	2	1	9	1	2	2	3	2	2	----- <i>Fomes pinicola</i> <i>Poria asiatica</i>
1	1	2	1	9	1	2	2	4	2	2	----- <i>Poria asiatica</i>
1	1	2	1	9	2	2	1	1	2	2	----- <i>Polyporus adustus</i>
1	1	2	1	9	2	2	1	1	2	3	----- <i>Polyporus adustus</i>
1	1	2	1	9	2	2	2	1	1	2	----- <i>Polyporus betulinus</i>
1	1	2	1	9	2	2	2	1	2	2	Mat thin, cottony-woolly to woolly-floccose in newest growth, finally collapsed and felty; good growth on gallic acid agar, no growth on tannic acid agar; oidia usually present.----- <i>Polyporus adustus</i>
											Mat slightly raised, cottony-floccose, so thin as to be translucent, becoming collapsed, with scattered dots or balls of more compact mycelium; good growth on both gallic and tannic acid agars ----- <i>Polyporus betulinus</i>
											Mat all submerged or appressed or with some areas developing powdery to floccose or tufted mycelium; reverse in some isolates greenish-yellow; no growth or only a trace on gallic and tannic acid agars ----- <i>Polyporus dichrous</i>
1	1	2	1	9	2	2	2	1	2	3	----- <i>Polyporus adustus</i>
1	1	2	1	9	2	2	2	2	1	1	----- <i>Fomes roseus</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—Continued

Hos	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	1	2	1	9	2	2	2	2	1	2	Mat pale pink; fruiting surface diffuse, granular to irregularly pored..... <i>Fomes roseus</i>
											Mat white, fruit bodies distorted mushrooms with gills..... <i>Pleurotus ostreatus</i>
											Mat white, fruiting in minutely pored areas on surface..... <i>Poria xantha</i>
											Mat white, fruiting over small compact balls usually grown against side wall of Petri dish, pored..... <i>Polyporus betulinus</i>
											Mat white, fruit bodies with gills, normal or distorted..... <i>Schizophyllum commune</i>
1	1	2	1	9	2	2	2	2	2	2	Mat cottony-woolly, usually uniform in appearance; diameter on gallic acid agar 1.5–4.0 cm., on tannic acid agar trace to 3.0 cm.. <i>Fomes pinicola</i>
											Mat appressed, woolly-felty, with slightly raised tufts, arranged in more or less concentric lines, producing zonate appearance; no growth on gallic or tannic acid agars..... <i>Pleurotus ostreatus</i>
											Mat slightly raised, cottony-floccose, so thin as to be translucent, becoming collapsed, with scattered dots or balls of more compact mycelium; good growth on both gallic and tannic acid agars..... <i>Polyporus betulinus</i>
											Mat all submerged or appressed or with some areas developing powdery to floccose or tufted mycelium; reverse in some isolates greenish-yellow; no growth or only a trace on gallic and tannic acid agars..... <i>Polyporus dichrous</i>
											Mat raised in newest growth, cottony, then collapsed, leaving thin film on agar; good growth on gallic acid agar, no growth on tannic acid agar..... <i>Poria xantha</i>
											Mat raised woolly, with scattered more or less compact lumps; good growth on both gallic and tannic acid agars..... <i>Schizophyllum commune</i>
1	1	2	1	9	2	2	2	3	1	2	..... <i>Trametes sepium</i>
											..... <i>Trametes serialis</i>
1	1	2	1	9	2	2	2	3	2	2	Mat cottony-woolly, usually uniform in appearance; diameter on gallic acid agar 1.5–4.0 cm., on tannic acid agar trace to 3.0 cm. diameter..... <i>Fomes pinicola</i>
											Mat raised, loosely arranged, tangled, cottony; no growth on gallic and tannic acid agars..... <i>Pleurotus ulmarius</i>
											Mat raised, cobwebby-cottony to plumose, becoming collapsed and appressed in older part; good growth on gallic acid agar, no growth on tannic acid agar..... <i>Polyporus subcartilagineus</i>
											Mat appressed downy in newest growth, so thin as to be translucent, soon developing zones in which mycelium is slightly raised, woolly, opaque; good growth on gallic acid agar, trace on tannic acid agar..... <i>Trametes sepium</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—Continued

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	1	2	1	9	2	2	2	4	2	2	----- <i>Pleurotus ulmarius</i>
1	1	2	2	1	2	2	2	2	1	2	----- <i>Polyporus fibrillosus</i>
1	1	2	2	9	1	1	2	2	2	2	----- <i>Polyporus sulphureus</i>
1	1	2	2	9	1	2	2	2	2	2	----- <i>Polyporus sulphureus</i>
1	1	2	2	9	2	2	2	1	2	3	----- <i>Polyporus Tulipiferae</i>
1	1	2	2	9	2	2	2	2	2	3	----- <i>Polyporus Tulipiferae</i>
1	1	2	4	9	2	2	1		2	1	----- <i>Coniophora puteana</i>
1	1	2	4	9	2	2	1		2	2	----- <i>Coniophora puteana</i>
1	1	2	4	9	2	2	2		2	1	----- <i>Coniophora puteana</i>
1	1	2	4	9	2	2	2		2	2	----- <i>Coniophora puteana</i>
1	2	1	1	5	1	2	2		2	3	----- <i>Ganoderma lucidum</i>
1	2	1	1	5	2	2	1	2	2	1	----- <i>Polyporus graveolens</i>
1	2	1	1	5	2	2	1	2	2	2	----- <i>Polyporus graveolens</i>
1	2	1	1	5	2	2	2	1	2	2	----- <i>Fomes fomentarius</i>
1	2	1	1	5	2	2	2	2	2	2	----- <i>Ganoderma applanatum</i>
											----- <i>Ganoderma lobatum</i>
1	2	1	1	5	2	2	2	4	2	2	----- <i>Ganoderma applanatum</i>
1	2	1	1	6	1	2	2	1	2	2	----- <i>Polyporus arcularius</i>
											----- <i>Polyporus brumalis</i>
1	2	1	1	6	1	2	2	1	2	3	----- <i>Polyporus arcularius</i>
											----- <i>Polyporus brumalis</i>
1	2	1	1	6	1	2	2	2	2	2	----- <i>Polyporus brumalis</i>
1	2	1	1	6	1	2	2	2	2	3	----- <i>Polyporus brumalis</i>
1	2	1	1	6	2	2	1	4	2	2	----- <i>Polyporus squamosus</i>
1	2	1	1	6	2	2	2	1	2	2	----- <i>Polyporus brumalis</i>
											----- <i>Polyporus arcularius</i>
1	2	1	1	6	2	2	2	1	2	3	----- <i>Polyporus arcularius</i>
											----- <i>Polyporus brumalis</i>
1	2	1	1	6	2	2	2	2	2	1	----- <i>Daedalea confragosa</i>
											----- <i>Polyporus tuberaster</i>
1	2	1	1	6	2	2	2	2	2	2	Mat "buffy brown" to "olive-brown" in small areas and in lines traversing white mat; thonglike strands grown through agar conspicuous from lower side
											----- <i>Collybia radicata</i>
											Mat white, with patches of "pinkish cinnamon", "cinnamon", and "snuff brown", to "bister", newer growth downy to fine woolly, becoming felty, to chamoislike, and crustose in colored areas
											----- <i>Daedalea confragosa</i>
											Mat white with scattered areas of "light vinaceous- cinnamon", "vinaceous-cinnamon", "cinnamon- buff", "tawny-olive", and "sandal brown", coalescing until whole surface is colored and mottled, cottony to woolly
											----- <i>Polyporus brumalis</i>
1	2	1	1	6	2	2	2	2	2	3	----- <i>Polyporus brumalis</i>
1	2	1	1	6	2	2	2	3	2	1	----- <i>Polyporus tuberaster</i>
1	2	1	1	9	1	2	2	2	2	1	----- <i>Polyporus cinnabarinus</i>
1	2	1	1	9	1	2	2	2	2	2	----- <i>Lentinus tigrinus</i>
1	2	1	1	9	2	1	2	2	1	2	----- <i>Polyporus rutilans</i>

KEY FOR THE IDENTIFICATION OF CULTURES OF  
WOOD-ROTTING FUNGI—Continued

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	2	1	1	9	2	1	2	2	2	2	----- <i>Pholiota adiposa</i>
											----- <i>Polyporus rutilans</i>
1	2	1	1	9	2	1	2	3	2	2	----- <i>Pholiota adiposa</i>
1	2	1	1	9	2	2	1	4	2	2	----- <i>Polyporus squamosus</i>
1	2	1	1	9	2	2	2	1	2	2	----- <i>Fomes fomentarius</i>
1	2	1	1	9	2	2	2	2	1	2	----- <i>Fomes scutellatus</i>
1	2	1	1	9	2	2	2	2	2	1	----- <i>Daedalea confragosa</i>
											----- <i>Polyporus tuberaster</i>
1	2	1	1	9	2	2	2	2	2	2	Mat "buffy brown" to "olive-brown" in small areas and in lines traversing white mat; thonglike strands grown through agar conspicuous from lower side
											----- <i>Collybia radicata</i>
											Mat white, with patches of "pinkish cinnamon", and "snuff brown", to "bister", newer growth downy to fine woolly, becoming felty to chamoislike and crustose in colored areas
											----- <i>Daedalea confragosa</i>
											Mat white, becoming "cream-buff", to "cinnamon-brown" in isolated areas, at first appressed cottony, becoming slightly raised, floccose to cottony-woolly
											----- <i>Fomes scutellatus</i>
1	2	1	1	9	2	2	2	3	1	2	----- <i>Fomes scutellatus</i>
1	2	1	1	9	2	2	2	3	2	1	----- <i>Polyporus tuberaster</i>
1	2	1	1	9	2	2	2	3	2	2	----- <i>Fomes scutellatus</i>
1	2	1	2	2	2	2	2	2	1	1	----- <i>Polyporus gilvus</i>
1	2	1	2	2	2	2	2	2	1	2	----- <i>Polyporus gilvus</i>
1	2	1	2	2	2	2	2	3	1	1	----- <i>Poria obliqua</i>
1	2	1	2	2	2	2	2	3	1	2	----- <i>Poria obliqua</i>
1	2	1	2	2	2	2	2	4	2	1	Setae numerous in some isolates, rare in others, 30.0–67.0 × 4.5–7.0μ; expansions on brown aerial hyphae up to 7.5μ diameter, occurring singly or in series
											----- <i>Fomes Pini</i>
											Setae or setal hyphae fairly numerous, 4.5–6.0μ diameter, up to 200μ long; no pseudoparenchymatous layer
											----- <i>Poria ferruginosa</i>
											Setal hyphae fairly numerous, 4.5–9.0μ diameter, up to 300μ long; pseudoparenchymatous layer composed of brown hyphae with interlocking short branches
											----- <i>Polyporus glomeratus</i>
1	2	1	2	5	2	2	2	1	2	1	----- <i>Fomes igniarius</i> var. <i>laevigatus</i>
1	2	1	2	5	2	2	2	2	2	1	----- <i>Fomes igniarius</i>
											<i>F. igniarius</i> var. <i>laevigatus</i>
1	2	1	2	5	2	2	2	3	2	1	----- <i>Fomes igniarius</i>
1	2	1	2	5	2	2	2	4	2	1	----- <i>Armillaria mellea</i>
											<i>Fomes igniarius</i> var. <i>populinus</i>
1	2	1	2	5	2	2	2	4	2	2	----- <i>Armillaria mellea</i>
1	2	1	2	6	2	2	2	2	1	2	----- <i>Polyporus dryophilus</i> var. <i>vulpinus</i>
1	2	1	2	6	2	2	2	2	2	1	----- <i>Polyporus radiatus</i>
1	2	1	2	6	2	2	2	2	2	2	----- <i>Hymenochaete tabacina</i>
											<i>Polyporus dryophilus</i> var. <i>vulpinus</i>



# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—*Continued*

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	2	1	2	6	2	2	2	3	1	2	----- <i>Polyporus dryophilus</i> var. <i>vulpinus</i>
1	2	1	2	6	2	2	2	3	2	2	----- <i>Polyporus dryophilus</i> var. <i>vulpinus</i>
1	2	1	2	6	2	2	2	4	2	1	----- <i>Polyporus glomeratus</i>
1	2	1	2	7	2	2	2	4	2	1	----- <i>Fomes conchatus</i> <i>Fomes Pini</i>
1	2	1	2	7	2	2	2	4	2	2	----- <i>Trameles tenuis</i>
1	2	1	2	9	2	1	2	1	2	2	----- <i>Fomes annosus</i>
1	2	1	2	9	2	1	2	4	2	2	----- <i>Fomes annosus</i>
1	2	1	2	9	2	2	2	1	2	1	----- <i>Fomes igniarius</i> var. <i>laevigatus</i> <i>Hymenochaete corrugata</i>
1	2	1	2	9	2	2	2	1	2	2	----- <i>Hymenochaete corrugata</i>
1	2	1	2	9	2	2	2	2	1	2	----- <i>Polyporus cuticularis</i> <i>Polyporus dryophilus</i> var. <i>vulpinus</i>
1	2	1	2	9	2	2	2	2	2	1	Mat with conspicuous white border, then "cream-buff", "honey yellow", to "tawny-olive", raised, thick woolly, nodulose; odor fragrant; restricted to species of Rosaceae.----- <i>Fomes fulvus</i>
											Mat with white border, then "cream-buff", "Naples yellow", "mustard yellow", in some isolates "honey yellow", "tawny-olive", "buckthorn brown", downy to cottony-woolly, with or without crustose areas; odor of wintergreen in some isolates ----- <i>Fomes igniarius</i>
											Mat with white border, then "cream-buff", "chamois", "honey yellow", and "clay color", "tawny-olive". "sayal brown", slightly raised cottony in newest growth, then woolly to tufted felty, with or without crustose zones; odor of wintergreen strong ----- <i>Fomes igniarius</i> var. <i>laevigatus</i>
											Mat with white border, changing abruptly to "clay color", "yellow ocher", "buckthorn brown", later with brighter colors of "old gold", "mustard yellow", etc., at first appressed, downy to cottony, then cottony-floccose, and finally felty and tufted; restricted to <i>Robinia pseudoacacia</i> .----- <i>Fomes rimosus</i>
											Mat with scattered areas and lines "chamois" and "buckthorn brown", slightly raised, woolly; odor sweet.----- <i>Hymenochaete corrugata</i>
											Mat white to "colonial buff", and "chamois", and "honey yellow", deepest color in newest growth, raised, cottony to cottony-woolly, older part collapsed, woolly, usually on <i>Quercus</i> spp. ----- <i>Polyporus dryophilus</i>
											Mat white, becoming "snuff brown", "Saccardo's umber", and "sepia" over inoculum and along radiating lines, cottony to felty or velvety ----- <i>Polyporus gilvus</i>
											Mat white, changing to "colonial buff", "chamois" slightly raised, cottony-woolly, somewhat tufted, the older part "olive-ocher", "honey yellow", "Isabella color", appressed, short cottony; slight odor.----- <i>Polyporus radiatus</i>

KEY FOR THE IDENTIFICATION OF CULTURES OF  
WOOD-ROTTING FUNGI—*Continued*

H at	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oldia	Growth rate	Fruiting	Reverse	
1	2	1	2	9	2	2	2	2	2	2	Mat with conspicuous white border, then "cream-buff", "honey yellow" to "tawny-olive", raised, thick woolly, nodulose; odor fragrant; restricted to species of Rosaceae..... <i>Fomes fulvus</i>
											Mat with scattered areas and lines "chamois" and "buckthorn brown", slightly raised, woolly; odor sweet..... <i>Hymenochaete corrugata</i>
											Mat white to "colonial buff" and "Isabella color", to "tawny-olive" in some isolates, newest growth raised, loosely arranged, cottony, later appressed, woolly to subfelty..... <i>Polyporus cuticularis</i>
											Mat with white border, then "colonial buff", "chamois", "deep colonial buff", "honey yellow", and "Isabella color", usually paler in older part, raised, cottony to cottony-plumose, collapsed around inoculum, subfelty to skinlike.... <i>Polyporus dryophilus</i> var. <i>vulpinus</i>
											Mat white, becoming "snuff brown", "Saccardo's umber", and "sepia" over inoculum and along radiating lines, cottony to felty or velvety..... <i>Polyporus gilvus</i>
1	2	1	2	9	2	2	2	2	2	3	..... <i>Polyporus dryophilus</i>
1	2	1	2	9	2	2	2	3	1	1	..... <i>Polyporus cuticularis</i>
1	2	1	2	9	2	2	2	3	1	2	..... <i>Polyporus dryophilus</i> var. <i>vulpinus</i>
1	2	1	2	9	2	2	2	3	2	1	Mat white to "baryta yellow", "light cadmium", and "yellow ocher", erect, silky-cottony, zonate, later developing opaque, azonate areas, "cream-buff", "chamois", and "honey-yellow"..... <i>Fomes Everhartii</i>
											Mat with white border, then "cream-buff", "Naples yellow", "mustard yellow", in some isolates "honey yellow", "tawny-olive", "buckthorn brown", downy to cottony-woolly, with or without crustose areas; odor of wintergreen in some isolates..... <i>Fomes igniarius</i>
											Mat with white border, changing abruptly to "clay color", "yellow ocher", "buckthorn brown", later with brighter colors of "old gold", "mustard yellow", etc., at first appressed, downy to cottony, then cottony-floccose, and finally felty and tufted; restricted to <i>Robinia pseudoacacia</i> ..... <i>Fomes rimosus</i>
											Mat white to "colonial buff" and "Isabella color", to "tawny-olive" in some isolates, newest growth raised, loosely arranged, cottony, later appressed, woolly to subfelty..... <i>Polyporus cuticularis</i>
											Mat white to "colonial buff", "chamois", and "honey yellow", deepest color in newest growth, raised, cottony to cottony-woolly, older part collapsed, woolly; usually on <i>Quercus</i> spp. .... <i>Polyporus dryophilus</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—*Continued*

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	2	1	2	9	2	2	2	3	2	2	Mat with white border, then "colonial buff", "chamois" "deep colonial buff", "honey yellow", and "Isabella color", usually paler in older part, raised, cottony to cottony-plumose, collapsed around inoculum, sub-felty to skinlike. <i>Polyporus dryophilus</i> var. <i>vulpinus</i>
											Mat white with tinges of "tawny-olive" and "buckthorn brown" over inoculum, and after three to four weeks in scattered flecks or narrow zones, appressed, cottony. <i>Poria ferrea</i>
											Mat white at first, then "pinkish buff", "chamois", and "tawny-olive", raised to top of Petri dish in vicinity of inoculum and gradually sloping to level of agar at margin, thin woolly. <i>Poria punctata</i>
1	2	1	2	9	2	2	2	3	2	3	<i>Polyporus dryophilus</i>
											<i>Poria ferrea</i>
1	2	1	2	9	2	2	2	4	2	1	Mat white to "cream-buff" and "chamois", raised, thick woolly, or white to "mustard yellow", "primuline yellow", "chamois", and "honey yellow", to "olive-ocher", cottony, so thin as to allow color of agar to show through, or showing sectors of both types of growth. <i>Fomes conchatus</i>
											Mat white to "baryta yellow", "light cadmium", and "yellow ocher", erect, silky-cottony, zonate, later developing opaque, azonate areas, "cream-buff", "chamois", and "honey yellow"
											<i>Fomes Everhartii</i>
											Mat with white border, then "colonial buff", "old gold", "honey yellow", "Saccardo's umber", "cinnamon-brown", raised, thick woolly to plushlike, with radiating grooves; strong odor of wintergreen; restricted to <i>Populus</i> spp.
											<i>Fomes ignarius</i> var. <i>populinus</i>
											Mat with narrow white border, then "yellow ocher" to "snuff brown", "old gold", "Dresden brown", "Sudan brown", etc., the paler areas thin woolly-felty, the reddish brown areas velvety
											<i>Polyporus dryadeus</i>
											Mat patchy, white to "barium yellow", "wax yellow", "yellow ocher", and "olive-yellow", cottony to woolly, sometimes zonate; setal hyphae and cuticular cells usually present. <i>Polyporus glomeratus</i>
1	2	1	2	9	2	2	2	4	2	2	<i>Trametes tenuis</i>
1	2	2	1	8	2	2	1	2	2	2	<i>Collybia velutipes</i>
1	2	2	1	9	1	2	1	2	1	2	<i>Lenzites trabea</i>
1	2	2	1	9	1	2	1	2	2	2	<i>Lenzites saepiarina</i>
1	2	2	1	9	1	2	1	3	2	2	<i>Lenzites saepiarina</i>
1	2	2	1	9	1	2	1	4	2	2	<i>Lenzites saepiarina</i>
1	2	2	1	9	2	1	2	2	2	2	<i>Polyporus guttulatus</i>
1	2	2	1	9	2	2	1	2	2	2	<i>Collybia velutipes</i>
											<i>Lenzites saepiarina</i>
1	2	2	1	9	2	2	1	3	2	2	<i>Lenzites saepiarina</i>
1	2	2	1	9	2	2	1	4	2	2	<i>Lenzites saepiarina</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—Continued

Host	Color	Reaction	Septation	Spec. stru <sup>u</sup>	Chlamydo <sup>x</sup>	Conidia	Oidia	Growth rate	Fruiting	Reverse	
1	2	2	4	9	2	2	1	1	2	1	----- <i>Coniophora puteana</i>
1	2	2	4	9	2	2	1	1	2	2	----- <i>Coniophora puteana</i>
1	2	2	4	9	2	2	2	1	2	1	----- <i>Coniophora puteana</i>
1	2	2	4	9	2	2	2	1	2	2	----- <i>Coniophora puteana</i>
2	1	1	1	0	1	2	2	2	1	2	----- <i>Polyporus borealis</i>
2	1	1	1	0	1	2	2	3	1	2	----- <i>Polyporus borealis</i>
2	1	1	1	0	2	2	2	4	2	1	----- <i>Polyporus semipileatus</i>
2	1	1	1	0	2	2	2	4	2	2	----- <i>Polyporus semipileatus</i>
2	1	1	1	1	1	2	2	2	1	2	----- <i>Polyporus borealis</i>
2	1	1	1	1	1	2	2	3	1	2	----- <i>Polyporus borealis</i>
2	1	1	1	1	1	2	2	4	2	1	----- <i>Echinodontium tinctorium</i>
2	1	1	1	1	2	2	2	2	1	2	----- <i>Polyporus pargamensis</i>
2	1	1	1	1	2	2	2	2	2	2	----- <i>Polyporus abietinus</i>
											----- <i>Polyporus pargamensis</i>
2	1	1	1	5	2	2	2	2	2	1	----- <i>Ganoderma oregonense</i>
2	1	1	1	5	2	2	2	2	2	2	Fiber hyphae numerous; widely distributed, but rarely encountered on coniferous trees
											----- <i>Ganoderma applanatum</i>
											Fiber hyphae lacking; limited to western coniferous trees
											----- <i>Ganoderma oregonense</i>
											Fiber hyphae numerous; limited to eastern coniferous trees
											----- <i>Ganoderma Tsugae</i>
2	1	1	1	5	2	2	2	2	2	3	----- <i>Ganoderma Tsugae</i>
2	1	1	1	5	2	2	2	4	2	2	----- <i>Ganoderma applanatum</i>
2	1	1	1	6	2	2	2	2	2	2	----- <i>Collybia radicata</i>
2	1	1	1	7	2	2	2	2	2	2	----- <i>Polyporus volvatus</i>
2	1	1	1	7	2	2	2	3	2	2	----- <i>Polyporus volvatus</i>
2	1	1	1	7	2	2	2	4	1	2	----- <i>Polyporus amorphus</i>
2	1	1	1	7	2	2	2	4	2	2	----- <i>Polyporus amorphus</i>
2	1	1	1	9	1	2	2	1	1	2	----- <i>Polyporus hirsutus</i>
2	1	1	1	9	1	2	2	1	2	2	----- <i>Polyporus hirsutus</i>
2	1	1	1	9	1	2	2	1	2	3	----- <i>Polyporus versicolor</i>
2	1	1	1	9	1	2	2	2	1	2	----- <i>Fomes subroseus</i>
											----- <i>Polyporus hirsutus</i>
											----- <i>Polyporus hirsutus</i>
2	1	1	1	9	1	2	2	2	2	2	----- <i>Polyporus versicolor</i>
2	1	1	1	9	1	2	2	3	2	2	----- <i>Poria asiatica</i>
2	1	1	1	9	1	2	2	4	2	1	----- <i>Echinodontium tinctorium</i>
2	1	1	1	9	1	2	2	4	2	2	----- <i>Poria asiatica</i>
2	1	1	1	9	2	1	2	2	2	2	----- <i>Pholiota adiposa</i>
2	1	1	1	9	2	1	2	3	2	2	----- <i>Pholiota adiposa</i>
2	1	1	1	9	2	2	2	1	1	2	----- <i>Polyporus anceps</i>
											----- <i>Polyporus hirsutus</i>
2	1	1	1	9	2	2	2	1	2	2	Mat at first floccose-woolly, becoming patchy, with some areas raised, felty-woolly, intervening areas appressed, thin felty
											----- <i>Lenzites betulina</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—*Continued*

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
2	1	1	1	9	2	2	2	1	2	2	Mat woolly-felty to plasterlike, with large compact lumps..... <i>Polyporus hirsutus</i>
											Mat uniformly cottony-woolly to felty, frequently with minute papillae where mat is grown against side of Petri dish..... <i>Poria subacida</i>
2	1	1	1	9	2	2	2	1	2	3	Mat at first woolly-floccose, becoming appressed woolly-felty, with slightly raised reticulations..... <i>Daedalea unicolor</i>
											Mat raised, cottony-woolly in newer growth and remaining so in some areas, otherwise felty, with drops of exudate that may leave surface punctate..... <i>Polyporus versicolor</i>
											Mat uniformly cottony-woolly to felty, frequently with minute papillae grown against side of Petri dish..... <i>Poria subacida</i>
2	1	1	1	9	2	2	2	2	1	1	..... <i>Fomes roseus</i>
2	1	1	1	9	2	2	2	2	1	2	Mat pale pink, fruiting surface diffuse, granular to irregularly pored..... <i>Fomes roseus</i>
											Mat white, fruit bodies distorted mushrooms with gills..... <i>Pleurotus ostreatus</i>
											Mat white, pored fruiting surfaces formed on large lumps over inoculum and elsewhere..... <i>Polyporus hirsutus</i>
2	1	1	1	9	2	2	2	2	2	2	Mat usually becoming "buffy brown" to "olive-brown" in small areas and in lines traversing white mat; thonglike strands grown through agar conspicuous from lower side..... <i>Collybia radicata</i>
											Mat white, at first floccose-woolly, becoming patchy, with some areas raised, felty-woolly, intervening areas appressed, thin felty..... <i>Lenzites betulina</i>
											Mat white, appressed, woolly-felty, with slightly raised tufts arranged in more or less concentric lines, producing zonate appearance..... <i>Pleurotus ostreatus</i>
											Mat white, woolly-felty to plasterlike, with large compact lumps..... <i>Polyporus hirsutus</i>
											Mat white, uniformly cottony-woolly to felty, frequently with minute papillae grown against side of Petri dish..... <i>Poria subacida</i>
2	1	1	1	9	2	2	2	2	2	3	..... <i>Polyporus versicolor</i>
											..... <i>Poria subacida</i>
2	1	1	1	9	2	2	2	4	1	2	..... <i>Polyporus amorphus</i>
2	1	1	1	9	2	2	2	4	2	2	..... <i>Polyporus amorphus</i>
2	1	1	2	7	2	2	2	4	2	2	..... <i>Trametes tenuis</i>
2	1	1	2	9	1	1	2	3	2	2	..... <i>Polyporus Berkeleyi</i>
2	1	1	2	9	1	2	2	3	1	2	..... <i>Polyporus Berkeleyi</i>
2	1	1	2	9	1	2	2	3	2	2	..... <i>Polyporus montanus</i>
2	1	1	2	9	2	1	2	1	2	2	..... <i>Fomes annosus</i>
2	1	1	2	9	2	1	2	4	2	2	..... <i>Fomes annosus</i>
2	1	1	2	9	2	2	1	1	2	2	..... <i>Peniophora gigantea</i>

KEY FOR THE IDENTIFICATION OF CULTURES OF  
WOOD-ROTTING FUNGI—Continued

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
2	1	1	2	9	2	2	1	2	2	2	----- <i>Peniophora gigantea</i>
2	1	1	2	9	2	2	2	1	2	1	----- <i>Hymenochaete corrugata</i>
2	1	1	2	9	2	2	2	1	2	2	----- <i>Hymenochaete corrugata</i>
2	1	1	2	9	2	2	2	2	2	1	----- <i>Hymenochaete corrugata</i>
2	1	1	2	9	2	2	2	2	2	2	----- <i>Hymenochaete corrugata</i>
2	1	1	2	9	2	2	2	3	2	2	----- <i>Polyporus montanus</i>
											----- <i>Poria ferrea</i>
2	1	1	2	9	2	2	2	3	2	3	----- <i>Poria ferrea</i>
2	1	1	2	9	2	2	2	4	2	2	----- <i>Trametes tenuis</i>
2	1	1	3	8	1	2	2		2	2	----- <i>Polyporus resinosus</i>
2	1	1	3	8	1	2	2		2	3	----- <i>Polyporus resinosus</i>
2	1	1	3	8	2	2	2		2	2	----- <i>Polyporus resinosus</i>
2	1	1	3	8	2	2	2		2	3	----- <i>Polyporus resinosus</i>
2	1	1	3	9	1	2	1		2	2	----- <i>Poria albipellucida</i>
											----- <i>Poria cinerescens</i>
2	1	1	3	9	1	2	1	1	2	3	----- <i>Poria albipellucida</i>
											----- <i>Poria cinerescens</i>
2	1	1	3	9	1	2	2	1	2	2	----- <i>Polyporus resinosus</i>
											----- <i>Poria albipellucida</i>
											----- <i>Poria cinerescens</i>
2	1	1	3	9	1	2	2	1	2	3	----- <i>Polyporus resinosus</i>
											----- <i>Poria albipellucida</i>
											----- <i>Poria cinerescens</i>
2	1		3	9	1	2	2	2	2	2	----- <i>Lentinus lepideus</i>
2	1		3	9	2	2	2	1	2	2	----- <i>Polyporus resinosus</i>
2	1		3	9	2	2	2	1	2	3	----- <i>Polyporus resinosus</i>
2	1		3	9	2	2	2	2	2	2	----- <i>Lentinus Kauffmanii</i>
2	1		3	9	2	2	2	3	2	2	----- <i>Lentinus Kauffmanii</i>
2	1		4	1	2	2	2	2	2	2	----- <i>Stereum sanguinolentum</i>
2	1		4	1	2	2	2	3	2	2	----- <i>Stereum sanguinolentum</i>
2	1		4	9	2	2	2	2	2	2	----- <i>Stereum sanguinolentum</i>
2	1		4	9	2	2	2	3	2	2	----- <i>Stereum sanguinolentum</i>
2	1	2	1	4		1	2	2	2	2	----- <i>Poria carbonica</i>
2	1	2	1	4	1	1	2	3	2	2	----- <i>Poria carbonica</i>
2	1	2	1	9	1	1	2	4	2	2	----- <i>Fomes officinalis</i>
2	1	2	1	9	1	2	1	2	2	2	----- <i>Trametes americana</i>
2	1	2	1	9	1	2	1	3	2	2	----- <i>Trametes americana</i>
2	1	2	1	9	1	2	2	1	1	2	----- <i>Poria monticola</i>
2	1	2	1	9	1	2	2	1	1	2	----- <i>Poria monticola</i>
2	1	2	1	9	1	2	2	2	1	1	----- <i>Polyporus balsameus</i>
2	1	2	1	9	1	2	2	2	1	2	Mat pink, downy to floccose, fruiting over most of surface; chlamydospores rare, thin-walled, 10.5-24.0 × 6.0-7.5μ; basidiospores cylindric, curved, 6.0-8.0 × 1.5-2.0μ----- <i>Fomes subroseus</i>
											Mat white or "pinkish buff" and "cinnamon" in older part, cottony, becoming coarse plumose or tufted, or with aerial mycelium scanty, appressed, downy, forming waxy pored fruiting surface; chlamydospores buffy brown when mature, 7.5-16.5 × 4.5-9.0μ----- <i>Polyporus balsameus</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—*Continued*

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
2	1	2	1	9	1	2	2	2	1	2	Mat white, slightly raised, downy to cottony floccose or thin felty, with scattered small raised balls of compact mycelium with surface at first floccose, later compact and covered with pores; chlamydospores rare, hyaline, $10.5-19.5 \times 7.5-12.0\mu$ ; basidiospores cylindric, $6.0-9.0 \times 2.0-3.0\mu$ ----- <i>Polyporus palustris</i>
											Mat white or with tinge of pale "cartridge buff" to "cinnamon buff", cottony to woolly, forming foliose fruit bodies; chlamydospores numerous to rare, with walls slightly thickened, $8.0-19.5 \times 6.0-13.5\mu$ ; basidiospores oblong-ellipsoid, $4.5-6.0 \times 2.2-2.7\mu$ ----- <i>Poria monticola</i>
											Mat white, newest growth raised, cottony, then collapsed, leaving thin film on agar; chlamydospores rare or apparently lacking, $10.6-19.5 \times 6.0-7.5\mu$ ; basidiospores cylindric, slightly curved, $4.2-5.0 \times 1.2\mu$ ----- <i>Poria xantha</i>
2	1	2	1	9	1	2	2	2	2	1	----- <i>Polyporus balsameus</i>
2	1	2	1	9	1	2	2	2	2	2	Mat white, cottony-woolly; chlamydospores numerous in some isolates, usually limited to advancing zone, $6.0-18.0 \times 6.0-9.0\mu$ ----- <i>Fomes pinicola</i> (See immediately above) <i>Polyporus balsameus</i> <i>Poria monticola</i> <i>Poria xantha</i>
											Mat white or becoming "cream-buff", "chamois", and "honey yellow" over oldest parts, at first short cottony, later woolly, opaque; chlamydospores fairly numerous, $9.0-21.0 \times 7.5-12.0\mu$ ----- <i>Trameles americana</i>
2	1	2	1	9	1	2	2	3	1	1	----- <i>Polyporus balsameus</i>
2	1	2	1	9	1	2	2	3	1	2	----- <i>Polyporus balsameus</i>
2	1	2	1	9	1	2	2	3	2	1	----- <i>Polyporus balsameus</i>
2	1	2	1	9	1	2	2	3	2	2	Mat white, cottony-woolly; chlamydospores numerous in some isolates, usually limited to advancing zone, $6.0-18.0 \times 6.0-9.0\mu$ ----- <i>Fomes pinicola</i>
											Mat white or "pinkish buff" and "cinnamon" in older part, cottony, becoming coarse plumose or tufted, or with aerial mycelium scanty, appressed, downy; chlamydospores buffy brown when mature, $7.5-16.5 \times 4.5-9.0\mu$ ----- <i>Polyporus balsameus</i>
											Mat white, raised, cottony-floccose in newer growth, becoming more compact, at least in part; chlamydospores numerous, usually rough-walled, $7.5-21.0 \times 4.5-12.0\mu$ ; odor of iodoform strong ----- <i>Poria asiatica</i>
											Mat white or becoming "cream-buff", "chamois", and "honey yellow" over oldest parts, at first short cottony, later woolly, opaque; chlamydospores fairly numerous, $9.0-21.0 \times 7.5-12.0\mu$ ----- <i>Trameles americana</i>
2	1	2	1	9	1	2	2	4	2	2	----- <i>Fomes officinalis</i> <i>Poria asiatica</i>

# KEY FOR THE IDENTIFICATION OF CULTURES OF WOOD-ROTTING FUNGI—Continued

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
2	1	2	1	9	2	2	1	1	2	2	----- <i>Polyporus adustus</i>
2	1	2	1	9	2	2	1	1	2	3	----- <i>Polyporus adustus</i>
2	1	2	1	9	2	2	2	1	2	2	----- <i>Polyporus adustus</i> ----- <i>Polyporus dichrous</i>
2	1	2	1	9	2	2	2	1	2	3	----- <i>Polyporus adustus</i>
2	1	2	1	9	2	2	2	2	1	1	----- <i>Fomes roseus</i>
2	1	2	1	9	2	2	2	2	1	2	Mat pale-pink; fruiting surface diffuse, granular to irregularly pored.----- <i>Fomes roseus</i>
											Mat white; fruit bodies distorted mushrooms with gills.----- <i>Pleurotus ostreatus</i>
											Mat white, slightly raised, cottony-woolly, frequently with strands originating at inoculum and extending across surface or around edge of plate and expanding in plumelike structure usually bearing pored fruit bodies.----- <i>Poria Vaillantii</i>
											Mat white, fruiting in minutely pored areas on surface ----- <i>Poria xantha</i>
2	1	2	1	9	2	2	2	2	2	2	Mat cottony-woolly, usually uniform in appearance; diameter on gallic acid agar 1.5-4.0 cm., on tannic acid agar trace to 3.0 cm.--- <i>Fomes pinicola</i>
											Mat appressed, woolly-felty, with slightly raised tufts, arranged in more or less concentric lines, producing zonate appearance; no growth on gallic or tannic acid agars.----- <i>Pleurotus ostreatus</i>
											Mat all submerged or appressed or with some areas developing powdery to floccose or tufted mycelium; reverse in some isolates greenish-yellow; no growth or only a trace on gallic and tannic acid agars ----- <i>Polyporus dichrous</i>
											Mat white, slightly raised, cottony-woolly, frequently with strands originating at inoculum and extending across surface or around edge of plate and expanding in plumelike structures; good growth on gallic acid agar, no growth on tannic acid agar ----- <i>Poria Vaillantii</i>
											Mat raised in newest growth, cottony, then collapsed, leaving thin film on agar; good growth on gallic acid agar, no growth on tannic acid agar ----- <i>Poria xantha</i>
2	1	2	1	9	2	2	2	3	1	2	Mat at first downy, translucent, becoming woolly, opaque, mostly appressed but frequently with a zone of raised mycelium midway across colony; fruiting after five to six weeks over areas of newest growth, the pores broad; basidiospores cylindric, 10.8-13.5 × 3.6-4.5μ.----- <i>Trametes heteromorpha</i>
											Mat at first appressed, downy, soon developing zones in which mycelium is slightly raised, woolly, opaque, the surface becoming pebbled, and pored; basidio- spores not found in six weeks old cultures ----- <i>Trametes sepium</i>





KEY FOR THE IDENTIFICATION OF CULTURES OF  
WOOD-ROTTING FUNGI—Continued

Host	Color	Reaction	Septation	Spec. structures	Chlamydo-spores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
2	2	1	1	5	2	2	2	2	2	1	----- <i>Ganoderma oregonense</i>
2	2	1	1	5	2	2	2	2	2	2	Fiber hyphae numerous; widely distributed, but rarely encountered on coniferous trees
											----- <i>Ganoderma applanatum</i>
											Fiber hyphae lacking; limited to western coniferous trees
											----- <i>Ganoderma oregonense</i>
											Fiber hyphae numerous; limited to eastern coniferous trees
											----- <i>Ganoderma Tsugae</i>
2	2	1	1	5	2	2	2	2	2	3	----- <i>Ganoderma Tsugae</i>
2	2	1	1	5	2	2	2	4	2	2	----- <i>Ganoderma applanatum</i>
2	2	1	1	6	2	2	2	2	2	1	----- <i>Polyporus tuberaster</i>
2	2	1	1	6	2	2	2	2	2	2	----- <i>Collybia radicata</i>
2	2	1	1	6	2	2	2	3	2	1	----- <i>Polyporus tuberaster</i>
2	2	1	1	6	2	2	2	4	2	2	----- <i>Omphalia campanella</i>
2	2	1	1	9	1	2	2	2	1	2	----- <i>Polyporus cinnabarinus</i>
2	2	1	1	9	2	1	2	2	2	2	----- <i>Pholiota adiposa</i>
2	2	1	1	9	2	1	2	3	2	2	----- <i>Pholiota adiposa</i>
2	2	1	1	9	2	2	2	2	2	1	----- <i>Polyporus tuberaster</i>
2	2	1	1	9	2	2	2	2	2	2	----- <i>Collybia radicata</i>
2	2	1	1	9	2	2	2	3	2	1	----- <i>Polyporus tuberaster</i>
2	2	1	2	2	1	2	2	4	2	1	----- <i>Polyporus circinatus</i>
2	2	1	2	2	2	2	2	2	1	1	----- <i>Polyporus gilvus</i>
2	2	1	2	2	2	2	2	2	1	2	----- <i>Polyporus gilvus</i>
2	2	1	2	2	2	2	2	2	2	2	----- <i>Poria Weirii</i>
2	2	1	2	2	2	2	2	2	2	3	----- <i>Poria Weirii</i>
2	2	1	2	2	2	2	2	3	2	2	----- <i>Poria ferrugineo-fusca</i>
2	2	1	2	2	2	2	2	4	2	1	Setae numerous in some isolates, rare in others, 30.0–67.0 × 4.5–7.0μ; expansions on brown aerial hyphae up to 7.5μ diameter, occurring singly or in series
											----- <i>Fomes Pini</i>
											Setae rare or apparently lacking in some isolates, 52.0–60.0 × 7.5–9.0μ; globose swellings and chlamydosporelike bodies numerous, hyaline to brown
											----- <i>Polyporus circinatus</i>
											Setae or setal hyphae fairly numerous, 4.5–6.0μ diameter, up to 200μ long
											----- <i>Poria ferruginosa</i>
2	2	1	2	3	2	2	2	4	2	1	----- <i>Fomes nigrolimitatus</i>
2	2	1	2	5	2	2	2	4	2	1	----- <i>Armillaria mellea</i>
2	2	1	2	5	2	2	2	4	2	2	----- <i>Armillaria mellea</i>
2	2	1	2	6	2	2	2	2	2	2	----- <i>Hymenochaete tabacina</i>
2	2	1	2	6	2	2	2	4	2	1	----- <i>Poria tsugina</i>
2	2	1	2	7	1	2	2	4	2	1	----- <i>Polyporus circinatus</i>
2	2	1	2	7	2	2	2	4	2	1	----- <i>Fomes Pini</i>
											----- <i>Polyporus circinatus</i>
2	2	1	2	7	2	2	2	4	2	2	----- <i>Trametes tenuis</i>
2	2	1	2	9	2	1	2	1	2	2	----- <i>Fomes annosus</i>
2	2	1	2	9	2	1	2	4	2	2	----- <i>Fomes annosus</i>
2	2	1	2	9	2	2	2	1	2	1	----- <i>Hymenochaete corrugata</i>
2	2	1	2	9	2	2	2	1	2	2	----- <i>Hymenochaete corrugata</i>

KEY FOR THE IDENTIFICATION OF CULTURES OF  
WOOD-ROTTING FUNGI—*Concluded*

Host	Color	Reaction	Septation	Spec. structures	Chlamydospores	Conidia	Oidia	Growth rate	Fruiting	Reverse	
2	2	1	2	9	2	2	2	2	2	1	----- <i>Hymenochaete corrugata</i>
											----- <i>Polyporus gilvus</i>
2	2	1	2	9	2	2	2	2	2	2	----- <i>Hymenochaete corrugata</i>
											----- <i>Polyporus gilvus</i>
2	2	1	2	9	2	2	2	3	2	2	----- <i>Poria ferrea</i>
2	2	1	2	9	2	2	2	3	2	3	----- <i>Poria ferrea</i>
2	2	1	2	9	2	2	2	4	2	1	Mat "chamois", "raw sienna", "buckthorn brown", "cinnamon-brown", slightly raised, loosely arranged, cottony-woolly, thin enough to allow dark color of agar to show through, colors becoming duller with age, collapsed, thin felty; reverse sometimes green in narrow zone before brown color appears; bulbils usually present; limited to western conifers
											----- <i>Fomes nigrolimitatus</i>
											Mat with narrow white border, then "yellow ochre" to "snuff brown", "old gold", "Dresden brown", "Sudan brown", etc., the paler areas thin woolly-felty, the reddish brown areas velvety; no bulbils
											----- <i>Polyporus dryadeus</i>
											Mat white at margin, changing to "cream color", "honey yellow", "tawny-olive", the newest growth slightly raised, short cottony, becoming opaque woolly, tufted, with brittle crustose areas "Sac- cardo's umber", in color; no bulbils but interlocking brown hyphae forming pseudoparenchymatous layer usually present
											----- <i>Poria tsugina</i>
2	2	1	2	9	2	2	2	4	2	2	----- <i>Trameles tenuis</i>
2	2	2	1	9	1	2	1	2	1	2	----- <i>Lenzites trabea</i>
2	2	2	1	9	1	2	1	2	2	2	----- <i>Lenzites saepiarina</i>
2	2	2	1	9	1	2	1	3	2	2	----- <i>Lenzites saepiarina</i>
2	2	2	1	9	1	2	1	4	2	2	----- <i>Lenzites saepiarina</i>
2	2	2	1	9	2	1	2	2	2	2	----- <i>Polyporus guttulatus</i>
2	2	2	1	9	2	2	1	2	2	2	----- <i>Lenzites saepiarina</i>
2	2	2	1	9	2	2	1	3	2	2	----- <i>Lenzites saepiarina</i>
2	2	2	1	9	2	2	1	4	2	2	----- <i>Lenzites saepiarina</i>
2	2	2	1	9	2	2	2	3	2	2	----- <i>Merulius lacrymans</i>
2	2	2	2	9	1	2	2	1	2	1	----- <i>Polyporus Schweinitzii</i>
2	2	2	2	9	1	2	2	1	2	2	----- <i>Polyporus Schweinitzii</i>
2	2	2	2	9	1	2	2	2	2	1	----- <i>Polyporus Schweinitzii</i>
2	2	2	2	9	1	2	2	2	2	2	----- <i>Polyporus Schweinitzii</i>
2	2	2	2	9	2	2	1	4	2	2	----- <i>Merulius lacrymans</i>
2	2	2	2	9	2	2	2	1	2	1	----- <i>Polyporus Schweinitzii</i>
2	2	2	2	9	2	2	2	1	2	2	----- <i>Polyporus Schweinitzii</i>
2	2	2	2	9	2	2	2	2	2	1	----- <i>Polyporus mollis</i>
											----- <i>Polyporus Schweinitzii</i>
2	2	2	2	9	2	2	2	2	2	2	----- <i>Polyporus mollis</i>
											----- <i>Polyporus Schweinitzii</i>
2	2	2	3	9	1	2	2	4	2	1	----- <i>Stereum abietinum</i>
2	2	2	4	9	2	2	1	1	2	1	----- <i>Coniophora puteana</i>
2	2	2	4	9	2	2	1	1	2	2	----- <i>Coniophora puteana</i>
2	2	2	4	9	2	2	2	1	2	1	----- <i>Coniophora puteana</i>
2	2	2	4	9	2	2	2	1	2	2	----- <i>Coniophora puteana</i>

## Descriptions of Cultures

### *Armillaria mellea* Vahl ex Fries

KEY PATTERN: (1,2) 2 1 2 5 2 2 2 4 2 (1,2)

#### CULTURES EXAMINED:

CANADA.—New Brunswick: Copper Cliff, F2890. British Columbia: Cowichan Lake, on *Pseudotsuga taxifolia*, 8185; Vancouver, on *P. taxifolia*, 8559. UNITED STATES.—Washington: Northport, on *Larix* sp., F7100.

CULTURAL CHARACTERS: (Pl. I, Fig. 1; Pl. II, Figs. 1 to 3).

GROWTH CHARACTERS.—Growth very slow, radius 3.0–4.0 cm. in six weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mats of two types: (a) white (two weeks), the central part becoming "wood brown" (7.0YR 5.7/4.0) to "Hay's brown" (2.0YR 3.5/2.0) (three weeks), raised, velvety to plushlike, with some radiating, valleylike grooves; reverse unchanged or "snuff brown" (7.0YR 3.9/3.5) below inoculum, with rhizomorphs numerous, short, branched, or completely lacking; (b) white, appressed, downy, zonate, with large areas "snuff brown" (7.0YR 3.9/3.5), "burnt umber" (2.0YR 2.5/2.5) (two weeks), and "carb brown" (2.0YR 3.1/2.3) (four weeks), felty to crustose or lacquerlike, in part covered with whitish bloom and scattered erect tufts of pinkish-brown hyphae, reverse unchanged to "snuff brown" (7.0YR 3.9/3.5) below colored areas, with branched rhizomorphs concolorous or white. Odor none. On gallic and tannic acid agars diffusion zones strong, no growth or only a trace on gallic acid agar, trace to 1.0 cm. on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with simple septa, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, frequently buffy-brown in older cultures; (b) hyphae in erect tufts buffy-brown, covered with minute hairlike projections. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) cuticular cells, at first hyaline with contents staining in phloxine, then apparently empty, with walls buffy-brown, closely packed to form pseudoparenchymatous layer.

TYPE OF ROT: soft white butt and root rot of coniferous and broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Benton and Ehrlich (28), Campbell (39), Cartwright and Findlay (54, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Edgecombe (69), Hamada (81), Reitsma (129).

Cultures of *Armillaria mellea* may be readily recognized on the basis of macroscopic appearance alone, their reddish-brown crustose areas, their rhizomorphs, and the frequent luminosity of young, actively-growing colonies providing a group of characters unique among the species studied. One of the key patterns coincides with that for *Fomes igniarius* var. *populinus*, but the distinctive wintergreen odor of this species readily separates it from *Armillaria mellea*.

### *Collybia radicata* Relh. ex Fries

KEY PATTERN: (1,2) (1,2) 1 1 (6,9) 2 2 2 2 2 2

#### CULTURES EXAMINED:

CANADA.—Quebec: Mt. Burnett, F3461. UNITED STATES.—F2934.

CULTURAL CHARACTERS: (Pl. I, Fig. 2; Pl. II, Figs. 4 to 6).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in four weeks. Advancing zone even, appressed in narrow zone, with fibers widely separated, making it difficult to see limit of growth. Mat white and remaining so, or after two to six weeks becoming "buffy brown" (9.0YR 4.6/3.5) to "olive-brown" (8.0YR 3.8/2.0) in small areas at edge and in lines traversing the white mat, raised, downy to cottony to cottony-woolly and somewhat spongy, later collapsed, felty, the colored areas crustose. Reverse unchanged, with conspicuous

thonglike strands of compact mycelium grown from surface down through the agar. Odor strong, penetrating. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, diameter 1.5–2.0 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5–3.0 (–6.0)  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, frequently branched; (b) crustose areas and thonglike strands composed of "intermeshing hyphae which form a homogeneous tissue without interhyphal space", that is, a pseudoparenchymatous layer. *Submerged mycelium*: hyphae as in advancing zone.

**TYPE OF ROT**: white rot of roots, presumably of broad-leaved and coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Campbell (40).

Cultures of this species that develop no pseudoparenchymatous layer and so remain white have a key pattern identical with those of several other species, all of which differ from *Collybia radicata* in the occurrence of numerous fiber hyphae. Cultures of *Collybia radicata* that form a pseudoparenchymatous layer and become colored have a key pattern like those for *Daedalea confragosa*, *Polyporus brumalis*, and *P. tuberaster*. The thonglike strands extending from the surface down through the agar occur only in *Collybia radicata*, and serve to distinguish this species from the other similar species.

**Collybia velutipes** Curt. ex Fries

**KEY PATTERN**: 1 2 2 1 (8,9) 2 2 1 2 2 2

**CULTURES EXAMINED**:

CANADA.—Ontario: Ottawa, on *Tilia* sp., F1598. GERMANY.—Eberswalde, on broad-leaved tree, F1294.

**CULTURAL CHARACTERS**: (Pl. I, Fig. 3; Pl. II, Figs. 7 to 9).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white (one week), the older part becoming "colonial buff" (6.0Y8.5/5.5) to "chamois" (2.0Y7.5/5.8) (two weeks), to "honey yellow" (2.0Y6.7/6.2) and "Saccardo's umber" (9.0YR3.8/3.5) (three to six weeks), the color gradually extending over colony but always mixed with white so that color appears pale, at first slightly raised, cottony with farinaceous surface, later appressed, thin woolly with minute tufts, approaching crustose in brown areas around inoculum. Reverse unchanged. Odor strong, suggesting iodoform, in cultures four weeks old, becoming fainter with age. On gallic and tannic acid agars no diffusion zones, no growth on either medium. (Davidson, Campbell, and Blaisdell report a very weak positive reaction on gallic acid agar.)

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5–3.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) oidia numerous, produced by the fragmentation of hyphae with simple septa formed as branches from nodose-septate hyphae, 2.2–3.0  $\mu$  diameter, of variable lengths; (c) lactiferous cells observed in F1598, deeply stained in phloxine and conspicuous in mounts, frequently with small projections, up to 9.0–15.0  $\mu$  diameter. *Submerged mycelium*: (a) hyphae as in advancing zone, up to 4.5  $\mu$  diameter; (b) lactiferous cells as in aerial mycelium.

**TYPE OF ROT**: white spongy rot of sapwood of living broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Brodie (36), Davidson, Campbell, and Blaisdell (64), Edgecombe (69), Heldmaier (82), Humphrey and Siggers (92), Zattler (152).

The restriction of oidium formation to hyphae with simple septa formed as branches from nodose-septate hyphae differentiates cultures of *Collybia velutipes* from those of *Lenzites saepiarum*, in which the nodose-septate hyphae undergo fragmentation to form oidia, and from *Merulius lacrymans*, in which

the whole mycelium reverts to a haploid condition, characterized by the presence of simple septa in all hyphae, and numerous oidia.

**Coniophora puteana** (Schum. ex Fries) Karst.

KEY PATTERN: (1,2) (1,2) 2 4 9 2 2 (1,2) 1 2 (1,2)

CULTURES EXAMINED:

CANADA.—Quebec: Champlain county, on *Picea mariana*, 10273, 10274; Eagle Depot, on *Abies balsamea*, 8546; Lac Humqui, on *A. balsamea*, F309. GERMANY.—9779.

CULTURAL CHARACTERS: (Pl. I, Fig. 4; Pl. II, Figs. 10 to 14).

GROWTH CHARACTERS.—Growth rapid, plates covered in two weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white and remaining so or with tinges of "cartridge buff" (3.0YR8.5/2.2), "colonial buff" (6.0YR8.5/5.5), and "deep colonial buff" (4.0Y7.7/5.5), the color deepest in newest growth, which is raised, cottony, tufted, the older part appressed, downy, with prominent radiating fibers or uniformly felty, frequently grown against sides of Petri dish and across lid. Reverse unchanged (two weeks) to "chamois" (2.0Y7.5/5.8), "honey yellow" (2.0Y6.7/6.2), and "cinnamon-brown" (5.0YR3.0/3.0). Odor strong, unpleasant. On gallic and tannic acid agars no diffusion zones, diameter 5.5–7.0 cm. on gallic acid agar, 1.5–2.0 cm. on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: (a) main hyphae hyaline, occasionally with a single clamp connection at a septum, usually with multiple clamp connections, frequently each producing a branch so that a whorl of branches is formed at a node, 6.0–12.0  $\mu$  diameter; (b) branches from main hyphae usually narrower, with inconspicuous simple septa, frequently branched, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) broad hyphae as in advancing zone, occasionally up to 15.0  $\mu$  diameter, observed only near edge of colony, soon empty and collapsed; (b) hyaline hyphae with simple septa as in advancing zone; (c) brown hyphae with simple septa, branched, with numerous knoblike or bulbous protuberances along walls, 2.2–4.5  $\mu$  diameter; (d) oidia observed in some isolates, especially in tufts of mycelium at edge of Petri dish, 2.2–6.0  $\mu$  diameter, variable in length. *Submerged mycelium*: (Agar almost liquefied below colony.) (a) hyaline hyphae with simple septa as in aerial mycelium; (b) crystals numerous, octahedral.

TYPE OF ROT: brown cubical rot of coniferous and broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Cartwright and Findlay (51, 52, 54, 56), Davidson and Campbell (63), Davidson, Campbell, and Blaisdell (64), Humphrey and Siggers (92), Kemper (95).

With four diagnostic characters that may vary, *Coniophora puteana* appears no less than 16 times in the key. The conspicuous multiple clamp connections and characteristic whorls of branches in the advancing zone separate it readily from the only other species, *Poria rufa*, which has a key pattern identical with any of those for *Coniophora puteana*.

**Daedalea confragosa** Bolt. ex Fries

KEY PATTERN: 1 2 1 1 (6,9) 2 2 2 2 2 (1,2)

CULTURES EXAMINED:

CANADA.—Quebec: Chelsea, on *Betula papyrifera*, 9210; Meach Lake, on *Fagus grandifolia*, F1577. Ontario: Constance Bay, on *Betula* sp., F5213; Ottawa, on *Acer* sp., 8997.

CULTURAL CHARACTERS: (Pl. I, Fig. 5; Pl. II, Figs. 15 to 17).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three weeks. Advancing zone even, hyaline and appressed in zone 1–2 mm. wide. Mat white, with patches of "pinkish cinnamon" (6.0YR6.5/5.5), "cinnamon" (5.0YR5.8/6.0), and "snuff brown"

(7.0YR3.9/3.5) (two to three weeks), to "bister" (4.5YR3.0/3.0) (four weeks), the newer growth downy to fine woolly and minutely lacunose, becoming felty to chamoislike, and crustose in colored areas. Reverse unchanged or "snuff brown" (7.0YR3.9/3.5) to "antique brown" (8.0YR4.2/2.5) in irregular patches. Odor in some isolates fairly strong at two to three weeks of age, suggesting pepper, disappearing with age. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace to 1.5 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, frequently branched, 1.5–3.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae numerous, with walls thick and refractive, hyaline or yellow in crustose areas, the lumina narrow or apparently lacking, occasionally branched, aseptate, curving and interwoven, 1.5–3.0  $\mu$  diameter; (c) hyphae from skinlike areas nodose-septate, with numerous short branches or projections, compactly arranged and interwoven with fiber hyphae to produce pseudoparenchymatous areas. *Submerged mycelium*: (a) hyphae as in advancing zone, frequently branched; (b) crystals short, needlelike.

**TYPE OF ROT**: white mottled rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67).

Cultures of *Daedalea confragosa* may fail to develop a definite pseudoparenchymatous layer, in which case the key pattern will be identical with those for *Fomes scutellatus* and *Collybia radicata*. In the key, mention is made of the macroscopic appearance of the cultures, but in addition it may be noted that *Fomes scutellatus* is usually limited to *Alnus* spp., and that fiber hyphae are lacking in cultures of *Collybia radicata* and numerous in cultures of the other species. Cultures of *Daedalea confragosa* that form a pseudoparenchymatous layer key also to *Polyporus tuberaster*, *P. brumalis*, and *Collybia radicata*. *Polyporus tuberaster* occurs very rarely, being known from Canada from only a few localities in Manitoba and Saskatchewan, and never in association with decay. Cultures of *Daedalea confragosa* differ from those of *Polyporus brumalis* in the extent of the colored areas on the surface, and from cultures of *Collybia radicata* in the presence of fiber hyphae, which are lacking in the latter species.

### ***Daedalea quercina* L. ex Fries**

**KEY PATTERN**: 1 1 2 1 9 1 2 2 2 (1,2) 2

#### **CULTURES EXAMINED:**

CANADA.—Ontario: Morton, on *Quercus* sp., F2278; Ottawa, on *Quercus* sp., F676. UNITED STATES.—North Carolina: F2158.

**CULTURAL CHARACTERS**: (Pl. I, Fig. 6; Pl. II, Figs. 18 to 24).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in four weeks. Advancing zone even, fibers appressed, closely arranged, radiating. Mat white, newest growth woolly floccose, becoming appressed, woolly-felty, with slightly raised reticulations, producing a "towelled" (Cartwright and Findlay) effect, frequently fruiting over raised tufts after five to six weeks. Reverse unchanged. Odor strong at three to four weeks, fruity. On gallic and tannic acid agars no diffusion zones, diameters 2.0–3.0 cm. on both media.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, frequently branched at and between septa, with occasional branches directed backward from posterior part of clamp connection, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, frequently with walls irregularly thickened and lumina relatively narrow, up to 6.0  $\mu$  diameter; (b) fiber hyphae numerous in cultures five to six weeks old, with walls thick and refractive, lumina narrow or apparently lacking, occasionally branched, aseptate, 2.2–3.0  $\mu$  diameter; (c) chlamydospores numerous, broadly ovoid, with thick walls, 12.0–18.0  $\mu$  diameter. *Fruit body*: (a) thin-walled hyphae and (b) fiber hyphae as in aerial mycelium; (c) basidia 4.5–6.0  $\mu$  diameter, bearing four sterigmata up to 6.0  $\mu$  long; (d) basidiospores hyaline, even, oblong-

ellipsoid to cylindric, slightly flattened on one side,  $6.0-8.0 \times 2.5-3.0\mu$ . *Submerged mycelium*: (a) nodose-septate hyphae up to  $8.0\mu$  diameter and (b) chlamydospores as in aerial mycelium; (c) crystals numerous, octahedral.

TYPE OF ROT: brown cubical rot of broad-leaved trees, especially *Quercus* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Cartwright and Findlay (51, 52, 53, 56), Davidson, Campbell, and Blaisdell (64), Humphrey and Siggers (92), Walek-Czernecka (150).

Cultures of *Daedalea quercina* have key patterns identical with those for a number of other species. In the inserted descriptive keys are noted differences in color and topography of the mats, and in chlamydospores and basidiospores, which should serve to distinguish the different species. Cultures of *Daedalea quercina* are similar to those of *Poria monticola* but the early formation of typical foliose fruit bodies by most cultures of the latter species makes its cultures readily recognizable.

### **Daedalea unicolor** (Bull.) Fries

KEY PATTERN: (1,2) 1 1 1 9 2 2 2 1 2 3

#### CULTURES EXAMINED:

CANADA.—Quebec: Iberville, F8022; Ile Jésus, on *Betula papyrifera*, 10202. Ontario: Lake of Bays district, on *B. papyrifera*, 10196.

CULTURAL CHARACTERS: (Pl. I, Fig. 7; Pl. II, Figs. 25 and 26).

GROWTH CHARACTERS.—Growth rapid, plates covered in two weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white, raised, loosely arranged, cottony-floccose, becoming collapsed, cottony-woolly in older part, with irregular thickenings giving lacunose effect, grown to top of Petri dish and extending over inner surface of lid in thick layer. Reverse unchanged for three to four weeks, then bleached. Odor strong, like *Psalliota campestris*. On gallic and tannic acid agars diffusion zones moderately strong, no growth on gallic acid agar, diameter 2.0–2.5 cm. on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate,  $1.5-4.5\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae numerous, with walls thick and refractive, lumina apparently lacking, aseptate, rarely branched,  $1.5-2.2\mu$  diameter. *Submerged mycelium*: hyphae as in advancing zone, up to  $6.0\mu$  diameter.

TYPE OF ROT: white rot of broad-leaved or, rarely, coniferous trees, frequently found decaying sapwood and causing cankers of living trees (Campbell (43)).

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64), Humphrey and Siggers (92).

The species having the same key patterns as *Daedalea unicolor* are difficult to separate in culture. The lacunose surface and growth of mycelium across the lid of the Petri dish in cultures of *D. unicolor* may be sufficiently distinctive to separate it from the other species in the group.

### **Echinodontium tinctorium** Ell. and Ev.

KEY PATTERN: 2 1 1 1 (1,9) 1 2 2 4 2 1

#### CULTURES EXAMINED:

CANADA.—British Columbia: Aleza Lake, on *Abies* sp., 16578; Queen Charlotte Islands, on coniferous tree, 16049; Trinity Valley, on *Tsuga*



*heterophylla*, 8740. UNITED STATES.—California: Strassburg, on *Abies concolor*, F620. Washington: Spirit Mountain, on *A. grandis*, F1157; Northport, on *Tsuga heterophylla*, F7423.

**CULTURAL CHARACTERS:** (Pl. I, Fig. 8; Pl. II, Figs. 27 to 30).

**GROWTH CHARACTERS.**—Growth very slow, radius 2.5–5.0 cm. in six weeks. Advancing zone even, appressed or with raised cottony mycelium extending to limit of growth. Mat white, appressed, farinaceous to felty, more or less completely overgrown with long tangled cottony mycelium, especially abundant against side of Petri dish above inoculum. Reverse "sandal brown" (7.0YR5.0/5.5) to "snuff brown" (7.0YR3.9/3.5), the colored diffusion zone frequently extending beyond edge of colony. Odor strong, disagreeable at first, suggestive of almonds at six weeks. On gallic acid agar diffusion zone strong, on tannic acid agar moderately strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 2.2–4.5  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, frequently with yellow to brown oily contents, with occasional swellings up to 7.5  $\mu$  diameter; (b) chlamydospores fairly numerous, with walls thin or slightly thickened, at first hyaline with contents stained in phloxine, later empty, broadly ovoid, 7.5–10.5  $\times$  6.5–9.0  $\mu$ ; (c) cystidia fairly numerous in most isolates, clavate or elongate-cylindric, frequently with single or moniliform swellings, with walls thick and lumina evident only in swollen parts, partially incrustated, up to 13.5  $\mu$  diameter. *Submerged mycelium:* (a) hyphae and (b) chlamydospores as in aerial mycelium.

**TYPE OF ROT:** brown stringy heart rot of living coniferous trees in western Canada and United States.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

Cystidia occur in cultures of *Echinodontium tinctorium*, but their appearance may be delayed in some isolates beyond the six weeks' period during which examinations are made. Therefore two key patterns are given for the species, one with cystidia, one without special structures, but neither of these coincides with the key pattern for any other species.

**Favolus alveolaris** (DC. ex Fries) Quél.

**KEY PATTERN:** 1 1 1 1 9 2 2 2 3 2 (2,3)

**CULTURES EXAMINED:**

CANADA.—Quebec: Chelsea, on *Ulmus americana*, F628. Ontario: Ottawa, on branch of broad-leaved tree, F1338.

**CULTURAL CHARACTERS:** (Pl. I, Fig. 9; Pl. II, Figs. 31 and 32).

**GROWTH CHARACTERS.**—Growth slow, plates covered in six weeks. Advancing zone even or somewhat bayed, hyaline and appressed in narrow zone. Mat white, appressed, fine woolly or subfelty. Reverse unchanged or bleached after three to four weeks. Odor slight. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, diameter up to 1.0 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae nodose-septate, 1.5–3.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae numerous, with walls thick and refractive, lumina apparently lacking, aseptate, branched, curving and interwoven, 1.0–1.5  $\mu$  diameter. *Submerged mycelium:* hyphae as in advancing zone.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

Cultures of *Favolus alveolaris* that do not bleach the agar have a key pattern identical with that of *Fomes Ellisianus*. The latter is specific to *Shepherdia*

*argentea*, which fact will probably serve to differentiate between these two species.

**Fomes annosus** (Fries) Cooke

KEY PATTERN: (1,2) (1,2) 1 2 9 2 1 2 (1,4) 2 2

CULTURES EXAMINED:

CANADA.—British Columbia: Oyster River, on log, 8273, on *Pseudotsuga taxifolia*, 8429, on *Tsuga heterophylla*, 8415; Queen Charlotte Islands, on *Picea sitchensis*, 10735; Royston, on rotted log, 8239. CZECHOSLOVAKIA.—F8061. ENGLAND.—Norfolk, on *Larix* sp., 9260. GERMANY.—Eberswalde, on conifer, F1288. HOLLAND.—Baarn, F6326, F6327.

CULTURAL CHARACTERS: (Pl. I, Fig. 10; Pl. II, Figs. 33 to 35).

GROWTH CHARACTERS.—Cultures of two types: (a) growth rapid, plates covered in two weeks. Advancing zone even, hyaline, appressed. Mat white, with patches of "cream-buff" (3.0Y8.3/4.5), "chamois" (2.0Y7.5/5.8), and "honey yellow" (2.0Y6.7/6.2) in some isolates after two to four weeks, the newer growth slightly raised, sparse downy to floccose-cottony, becoming collapsed and appressed, thin felty, with farinaceous surface; (b) growth very slow, radius 3.0 cm. or less in six weeks. Advancing zone even or bayed, felty aerial mycelium uniform to limit of growth. Mat white, appressed, felty with somewhat farinaceous surface. Reverse unchanged or with zones of "honey yellow" (2.0Y6.7/6.2). Odor none in some isolates, faintly of lemon in others, strong and disagreeable in the remainder. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae with simple septa or rare clamp connections, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, 2.2–6.0  $\mu$  diameter; (b) conidiophores numerous in most isolates, consisting of erect stalks, simple or branched, 4.5–7.5  $\mu$  diameter, swollen to form globose heads, 7.5–22.0  $\mu$  diameter, bearing conidia on slender pointed sterigmata over the distal end of the head and occasionally along the stalk; (c) conidia very numerous, hyaline, even, subglobose to ovoid, apiculate, 4.5–7.5 (–10.5)  $\times$  3.0–6.0  $\mu$ . *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white pocket rot of coniferous or, rarely, broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Campbell (42), Cartwright and Findlay (51, 54, 56), Davidson, Campbell, and Blaisdell (64), Humphrey and Siggers (92), Roll-Hansen (135).

In *Fomes annosus* there is the possibility of variation in three of the characters used in the identification of cultures, which results in this species appearing eight times in the key. In spite of this its cultural characters are unique and it is not to be confused with any other species included in the key. One of the variations reported is that in growth rate, some isolates growing rapidly, others very slowly, and some growing rapidly at one time of planting, slowly at another. It seems probable that the rapid growth is more usual and that the slow growth is the result of some local adverse condition. An additional variation not considered in the key is the possible presence of clamp connections. They were observed in a few isolates, but they occurred only rarely, and always in hyphae in which most of the septa were simple. Roll-Hansen (135) has reported a similar observation.

**Fomes conchatus** (Pers. ex Fries) Gill.

KEY PATTERN: 1 2 1 2 (7,9) 2 2 2 4 2 1

## CULTURES EXAMINED:

CANADA.—Quebec: Kingsmere, on log, 9219; Montreal, on log of broad-leaved tree, F2571; Pointe Platon, on *Crataegus* sp., 10159. Ontario: Lake Timagami, on fallen log, F1403; Ottawa, on stump, F1381. UNITED STATES.—on *Fraxinus americana*, F639.

CULTURAL CHARACTERS: (Pl. I, Fig. 11; Pl. II, Figs. 36 to 38).

GROWTH CHARACTERS.—Growth very slow, radius 4.5–7.5 cm. in six weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mats of three types: (a) white to "mustard yellow" (2.0Y7.8/7.5), "primuline yellow" (1.5Y7.5/9.5), "chamois" (2.0Y 7.5/5.8), and "honey yellow" (2.0Y6.7/6.2) (two weeks) to "deep colonial buff" (4.0Y7.7/5.5) and "olive-ocher" (3.0Y6.8/6.5) (three weeks), with little subsequent change in color, slightly raised, cottony, so thin that color of darker agar shows through; (b) white to "cream-buff" (3.0Y8.3/4.5) and "chamois" (2.0Y7.5/5.8), raised, thick woolly, opaque; (c) sectoried, with varying amounts of opaque woolly mycelium as in (b) and thin mycelium as in (a). Reverse unchanged below newer growth, then "buckthorn brown" (8.0YR4.8/6.5), "sandal brown" (7.0YR5.0/5.5) to "cinnamon-brown" (5.0YR3.0/3.0) and "snuff brown" (7.0YR 3.9/3.5), with greenish tinge approaching "citrine-drab" (5.5Y5.0/2.8) sometimes observed. Odor none. On gallic and tannic acid agars diffusion zones weak to strong, no growth on gallic acid agar, trace to 2.0 cm. on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with simple septa, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) colored hyphae, with greenish-yellow walls and contents hyaline or dark brown, septate, branched, 2.2–6.0  $\mu$  diameter. *Submerged mycelium*: (a) hyphae hyaline to brown, frequently branched and septate, 3.0–7.5  $\mu$  diameter; (b) swellings on hyphae, usually terminal, up to 20  $\mu$  diameter, numerous in some isolates, rare or apparently lacking in others.

TYPE OF ROT: white rot of broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Campbell (42), Davidson, Campbell, and Blaisdell (64).

Cultures of *Fomes conchatus* with swollen cells have a key pattern identical with that for *F. Pini*, but the swellings on the hyphae of the latter species are distinctive, usually occurring in series in brown thick-walled aerial hyphae, while those of *F. conchatus* are single, usually terminal, and restricted to submerged mycelium. Cultures without these special structures coincide in the key with a number of other species, which must be separated on color and topographical differences that cannot be incorporated in a numerical key. Therefore a descriptive key to this group has been inserted.

**Fomes connatus** (Weinm.) Gill.

KEY PATTERN: 1 1 1 2 (7,9) 2 2 2 4 2 2

## CULTURES EXAMINED:

CANADA.—Quebec: Chelsea, on *Acer* sp., 10253; on broad-leaved tree, 10254. UNITED STATES.—Massachusetts: Harvard Forest, on *Ulmus* sp., F7990.

CULTURAL CHARACTERS: (Pl. I, Fig. 12; Pl. II, Figs. 39 and 40).

GROWTH CHARACTERS.—Growth very slow, radius 7.0 cm. or less in six weeks. Advancing zone even, appressed, hyaline. Mat white, at first limited to cottony ball over inoculum, gradually with appressed growth on agar, more or less completely covered with long, tangled, cottony mycelium. Reverse unchanged. On gallic and tannic acid agars diffusion zones weak, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, with simple septa, frequently branched, 2.2–4.5 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae rare. *Submerged mycelium*: thin-walled hyphae as in advancing zone.

**TYPE OF ROT**: soft white rot of broad-leaved trees, especially *Acer* spp.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Campbell (41), Davidson, Campbell, and Blaisdell (64).

The key patterns for *Fomes connatus* coincide with those for *Trameles tenuis*, but the latter species, while white at first, invariably develops scattered yellow to brown areas, and its appressed farinaceous to thin felty mat is completely unlike the long tangled cottony growth of *Fomes connatus*.

### **Fomes Ellisianus** Anderson

**KEY PATTERN**: 1 1 1 1 9 2 2 2 (2,3) 2 2

**CULTURES EXAMINED**:

CANADA.—Saskatchewan: Saskatoon, on *Shepherdia argentea*, F7364, F7520.

**CULTURAL CHARACTERS**: (Pl. I, Fig. 13; Pl. II, Figs. 41 to 43).

**GROWTH CHARACTERS.**—Growth moderately slow to slow, plates covered in four to six weeks. Advancing zone even, hyaline and appressed for short distance, or with aerial mycelium uniform to limit of growth. Mat white, with "cream-buff" (3.0Y8.3/4.5) tinge over inoculum after four to six weeks, appressed, downy, becoming slightly more compact woolly around inoculum. Reverse unchanged or slightly darker. Odor none. On gallic and tannic acid agars diffusion zones weak to moderately strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5–4.5 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, frequently septate and branched, 1.5–3.0 $\mu$  diameter; (b) fiber hyphae with walls slightly thickened, lumina fairly broad but apparently empty, aseptate, branched, 2.2–4.5 $\mu$  diameter, rare to fairly numerous. *Submerged mycelium*: (a) nodose-septate hyphae as described above; (b) crystals numerous, needlelike or platelike.

**TYPE OF ROT**: white rot of *Shepherdia argentea*.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Baxter (8).

*Fomes Ellisianus*, known only from *Shepherdia argentea*, is considered by some students to be a form of *Fomes fraxinophilus*, which is usually confined to *Fraxinus* spp. Thus Baxter (8), as a result of comparative studies of the fruit bodies, the types of decays they caused, and the cultural characters of the two fungi, concluded that "they must be considered as a single species. *Fomes Ellisianus* Anderson is considered a form of *Fomes fraxinophilus* Pk." In the present study the presence of chlamydospores and early production of fruiting surfaces in culture served to distinguish *F. fraxinophilus* from *F. Ellisianus*, which lacks these characters. Pairing tests showed both species to be heterothallic, and of the tetrapolar type of interfertility. When monosporous mycelia from one collection of *F. fraxinophilus* were paired with those from another collection of the same species, mycelia bearing clamp connections were produced in every pairing, but complete lack of fertility was found when monosporous mycelia of *F. fraxinophilus* were paired with those of *F. Ellisianus*. This is a further indication that these constitute two distinct species.

*Fomes Ellisianus* falls with other species in the key, but its specificity to *Shepherdia argentea* is probably sufficient to separate it from them.

**Fomes Everhartii** (Ell. and Gall.) von Schrenk

KEY PATTERN: 1 2 1 2 9 2 2 2 (3,4) 2 1

## CULTURES EXAMINED:

CANADA.—Quebec: Iberville, on *Fagus grandifolia*, F3580. UNITED STATES.—Michigan: Atlanta, on *Quercus velutina*, F2336. Locality and host not known, F3044.

CULTURAL CHARACTERS: (Pl. I, Fig. 14; Pl. II, Figs. 44 to 46).

GROWTH CHARACTERS.—Growth slow to very slow, plates covered in five to seven weeks. Advancing zone even, hyaline to white, aerial mycelium extending to limit of growth. Mat white to "baryta yellow" (4.0YR 8/7.0), "light cadmium" (2.0Y 7.8/9.5), and "yellow ochre" (10.0YR 6.8/9.0) (one to four weeks), erect, silky-cottony, the fibers straight and 'combed' in appearance, zonate, so thin as to allow dark color of agar to show through, "often with a greenish-yellow sheen", after four or five weeks developing opaque, azonate areas more or less extensive, "cream-buff" (3.0Y 8.3/4.5), "chamois" (2.0Y 7.5/5.8), and "honey-yellow" (2.0Y 6.7/6.2) in color. Reverse "buckthorn brown" (8.0YR 4.8/6.5), "cinnamon-brown" (5.0YR 3.0/3.0), and "mummy brown" (7.5YR 2.5/2.3), somewhat zonate. Odor none. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with simple septa, 2.2-4.5 (–6.0)  $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone but with walls hyaline to brown, sometimes with dense dark brown contents, branched, frequently septate, 2-6.0  $\mu$  diameter, occasionally compacted into small bulbil-like masses. *Submerged mycelium*: hyphae as in aerial mycelium.

TYPE OF ROT: "soft white or yellow flaky heart rot" of broad-leaved trees, usually *Quercus* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Baxter (7), Campbell (42), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Humphrey and Siggers (92).

From the key it will be observed that a number of species have key patterns identical with those for *Fomes Everhartii*. When once it has become familiar, the appearance of the mat composed of straight silky 'combed' fibers with a greenish-yellow sheen over the darker brown agar is sufficiently characteristic to separate it from other species.

**Fomes fomentarius** (L. ex Fries) Kickx

KEY PATTERN: 1 2 1 1 (5,9) 2 2 2 1 2 2

## CULTURES EXAMINED:

CANADA.—Quebec: Kingsmere, on *Betula lutea*, 9214; Lac Clair, on *Betula* sp., 11599. SCOTLAND.—Pitlochry, on *Betula* sp., F8038, F8039.

CULTURAL CHARACTERS: (Pl. I, Fig. 15; Pl. II, Figs. 47 and 48).

GROWTH CHARACTERS.—Growth rapid, plates covered in two weeks. Advancing zone even, with aerial mycelium uniform to limit of growth. Mat white with tinge of "cream-buff" (3.0Y 8.3/4.5) and "chamois" (2.0Y 7.5/5.8) (one week), to "light pinkish cinnamon" (7.0YR 7.5/4.5), "saval brown" (7.0YR 5.0/5.5), and "cinnamon-brown" (5.0YR 3.0/3.0) (two weeks), with little subsequent change in color but extension in size of colored areas, at first slightly raised, short cottony, then appressed, felty to chamoislike in colored areas, with some raised white woolly plateaus. Reverse unchanged. Odor none. On gallic and tannic acid agars diffusion zones moderately strong to strong, no growth on gallic acid agar, 1.0-2.0 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5-3.0 (–4.5)  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae numerous,

with walls thick and refractive, hyaline to buffy-brown, lumina narrow but evident, occasionally branched, aseptate, 1.5–3.0  $\mu$  diameter, closely interwoven in skinlike areas; (c) cuticular cells, closely packed and interwoven with fiber hyphae in pseudoparenchymatous layer, observed in only two isolates. *Submerged mycelium*: hyphae as in advancing zone.

**TYPE OF ROT:** white mottled rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Campbell (42), Cartwright and Findlay (55, 56), Davidson, Campbell, and Blaisdell (64), Fritz (74), Hilborn (83), Macdonald (102), Meyer (105).

In most isolates the tough chamoislike areas were composed of interwoven fiber hyphae only, but two cultures showed cuticular cells present in these areas, closely intermingled with the fiber hyphae. This character brings *Fomes fomentarius* close to the *Ganoderma* species, but the more rapid rate of growth of *Fomes fomentarius* separates it from these and other species in the key.

**Fomes fraxineus** (Bull. ex Fries) Cooke

**KEY PATTERN:** 1 1 1 1 (5,9) 1 2 2 2 (1,2) 2

**CULTURES EXAMINED:**

CANADA.—Quebec: Montreal, on *Ulmus* sp., F7519. UNITED STATES.—Virginia: F2161. Locality not known, on *Fraxinus* sp., F2022.

**CULTURAL CHARACTERS:** (Pl. III, Fig. 1; Pl. IV, Figs. 1 to 6).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, hyaline, appressed, silky. Mat white, cottony near margin, becoming felty and irregularly thickened, usually 'corroded' in appearance, sometimes with drops of exudate over surface, fruiting in some isolates after three to four weeks in organized pored areas or in inconspicuous groups of basidia scattered over surface. Reverse unchanged. Odor none. On gallic and tannic acid agars diffusion zones weak to moderately strong, no growth on gallic acid agar, trace to 1.5 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae nodose-septate, 1.5–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae numerous, walls thick, lumina narrow or apparently lacking, branched, aseptate, 1.5–4.5  $\mu$  diameter; (c) chlamydospores numerous, terminal and intercalary, globose to ovoid with walls noticeably thick and often appearing sculptured, 10.5–16.5  $\times$  7.5–12.0  $\mu$ ; (d) hyphae with tips branched dichotomously in staghorn or coralloid effect, observed in some isolates; (e) cuticular cells, hyaline, empty, forming a thin pseudoparenchymatous layer, observed in F7519 only. *Fruit body*: (a) basidia 7.5–10.5  $\mu$  diameter, each bearing four spores; (b) basidiospores hyaline, even, subglobose or broadly ovoid, apiculate, with conspicuous oil drop, 5.4–6.0  $\mu$  diameter, observed in large numbers in old cultures, apparently empty. *Submerged mycelium*: (a) nodose-septate hyphae and (b) chlamydospores as described above.

**TYPE OF ROT:** white rot of broad-leaved trees, especially *Fraxinus* spp.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Baxter (6), Campbell (42), Cartwright and Findlay (51, 55, 56), Davidson, Campbell and Blaisdell (64), Montgomery (107).

Cultures of *Fomes fraxineus* that form cuticular cells fall alone in the key, but the more numerous isolates lacking these have key patterns identical with those for several other species. The inserted descriptive keys mention topographical and microscopic differences that should make separation possible. The chlamydospores of *Fomes fraxineus*, with thick sculptured walls, are distinctive.

**Fomes fraxinophilus** (Peck) Sacc.

KEY PATTERN: 1 1 1 1 9 1 2 2 2 1 2

## CULTURES EXAMINED:

CANADA.—Quebec: Chelsea, on *Fraxinus americana*, F632. UNITED STATES.—Indiana: Greensfork, on *Fraxinus americana*, F1929. Michigan: Ann Arbor, on *F. americana*, F1931. New York: Newburgh, on *F. americana*, F1930. Locality and host unknown, F1932.

CULTURAL CHARACTERS: (Pl. III, Fig. 2; Pl. IV, Figs. 7 to 11).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, hyaline, appressed in narrow zone. Mat white, downy near margin, becoming compact felty around inoculum (one week), forming a uniform pellicle like soft chamois, which peels from agar readily (two weeks), with areas of raised velvety overgrowth, frequently along radii, usually bearing well-organized, poroid, waxy, fruiting surfaces (three to four weeks). Reverse unchanged to "pinkish buff" (9.0YR7.3/4.5). Odor none. On gallic and tannic acid agars diffusion zones moderately strong, no growth on gallic acid agar, 2.0–3.5 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae nodose-septate, frequently branched, with numerous anastomoses, 1.5–4.5 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae with thick walls, lumina narrow or apparently lacking, frequently branched and closely interwoven, 2.0–3.0 $\mu$  diameter; (c) basidiospores (from spore deposit) hyaline, even, ovoid, frequently truncate, walls fairly thick and refractive, 6.0–7.5  $\times$  4.5–6.0 $\mu$ . *Submerged mycelium*: (a) hyphae as in advancing zone; (b) chlamydo-spores few to numerous, terminal and intercalary, ovoid to elongate, fairly thin-walled, 9.0–18.0  $\times$  6.0–9.0 $\mu$ ; (c) crystals numerous, needlelike.

TYPE OF ROT: white mottled rot of broad-leaved trees, usually *Fraxinus* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Baxter (8), Campbell (42), Davidson, Campbell, and Blaisdell (64).

Cultures of *Fomes fraxinophilus* are distinguished from other species having an identical key pattern by the texture of the mat, the raised pored fruiting surfaces, which are frequently radically elongated, and the characteristic truncate spores. Secondary spores such as were described by Campbell (42) were not observed in the isolates studied. A note on the differences between cultures of *F. fraxinophilus* and *F. Ellisianus* is included under the latter species.

**Fomes fulvus** (Scop.) Gill.*Fomes pomaceus* (Pers.) Lloyd

KEY PATTERN: 1 2 1 2 9 2 2 2 2 2 (1,2)

## CULTURES EXAMINED:

Received from Centraalbureau voor Schimmelcultures, Baarn, Holland, F2283, F7310.

CULTURAL CHARACTERS: (Pl. III, Fig. 3; Pl. IV, Figs. 12 to 15).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in four weeks. Advancing zone even, white, raised woolly mycelium extending to limit of growth. Mat with conspicuous white border, then "cream-buff" (3.0Y8.3/4.5) to "chamois" (2.0Y7.5/5.8) (one week), to "honey yellow" (2.0Y6.7/6.2) (two weeks), to "tawny-olive" (8.0YR4.8/5.8) (four weeks), raised, thick woolly, 'nodulose' or 'lacunose', peeling readily from agar. Reverse unchanged or with scattered areas of "burnt sienna" (2.0YR3.2/7.0) after four weeks. Odor fragrant. On gallic and tannic acid agars diffusion zones moderately strong, no growth on gallic acid agar, trace on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, with inconspicuous simple septa; frequently branched, 1.5–4.5 $\mu$  diameter. *Aerial mycelium* (preparation turns red-brown in potassium hydroxide): (a) hyphae as in advancing zone; (b) colored hyphae, with walls slightly thickened and buffy-brown, with few inconspicuous simple septa, rarely branched, 1.5–2.2 $\mu$  diameter, with long tapering ends, frequently helicoid. *Submerged mycelium*: hyphae as in advancing zone, occasionally up to 6.0 $\mu$  diameter, with numerous septa.

**TYPE OF ROT**: white rot of "trees belonging to the order Rosaceae" according to Cartwright and Findlay (55).

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Baxter (8), Campbell (42), Cartwright and Findlay (55, 56), Davidson, Campbell, and Blaisdell (64), Fisher (72).

*Fomes fulvus* has a key pattern identical with those of several other species, of which *F. igniarius* is most difficult to separate from *F. fulvus*. The inserted descriptive key points out a number of differences, of which the occurrence of *F. fulvus* on members of the Rosaceae only may be most helpful.

**Fomes igniarius** (L. ex Fries) Gill.

**KEY PATTERN**: 1 2 1 2 (5,9) 2 2 2 (2,3) 2 1

**CULTURES EXAMINED**:

CANADA.—Nova Scotia: Kentville, on *Fagus grandifolia*, F5028. Quebec: Ste. Anne de la Pocatière, on *Pyrus* sp., F6915. Ontario: Petawawa, on *Acer saccharum*, F7972, on *Betula lutea*, F7978, on *B. papyrifera*, F7980, on *Fagus grandifolia*, F7977, on *Ostrya virginiana*, F7974, on *Quercus rubra*, F7975; Radiant, on *Betula papyrifera*, F7403. British Columbia: Cowichan, on *Salix* sp., 8207; Duncan, on *Salix* sp., F1255; Saanichton, on *Cornus nuttallii*, 8204. ENGLAND.—F3310.

**CULTURAL CHARACTERS**: (Pl. III, Fig. 4; Pl. IV, Figs. 16 to 19).

**GROWTH CHARACTERS.**—Growth moderately rapid to slow, plates covered in four to five weeks. Advancing zone even, white, with raised aerial mycelium extending to limit of growth. Mat white with tinges of "cream-buff" (3.0YR 8.3/4.5), "chamois" (2.0Y 7.5/5.8), "Naples yellow" (3.0Y 7.8/5.0), and "mustard yellow" (2.0Y 7.8/7.5) (one week), remaining pale or assuming deeper colors of "honey yellow" (2.0Y 6.7/6.2), "olive-ocher" (3.0Y 6.8/6.5), and "tawny-olive" (8.0YR 4.8/5.8) (two weeks), to "buckthorn brown" (8.0YR 4.8/6.5) (three weeks), (a) downy at margin, to cottony and woolly in older parts, with whole surface finally covered with uniform woolly to felty mycelium, or (b) with some zones with scanty cottony mycelium, white to "honey yellow" (2.0Y 6.7/6.2) and "yellow ocher" (10.0YR 6.8/9.0) over dark brown crustose areas in agar, remainder of the mat cottony to felty, mycelium frequently heaped up at the edge of the crustose areas. Reverse unchanged to the end of two to four weeks, then patchy, with scattered lines and dots of "ochraceous-tawny" (6.0YR 4.9/6.3), "cinnamon-brown" (5.0YR 3.0/3.0), "mummy brown" (7.5YR 2.5/2.3), etc., finally all brown, usually with darker lines and dots, producing a marblelike effect. Odor of wintergreen slight in some isolates, lacking in others. On gallic and tannic acid agars diffusion zones weak to strong, no growth or only a trace on both media.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, with inconspicuous simple septa, frequently branched, 1.5–6.0 $\mu$  diameter. *Aerial mycelium* (peels readily from agar and turns brown in potassium hydroxide): (a) hyphae as in advancing zone; (b) fiber hyphae, with walls more or less thickened, greenish-yellow to brown, rarely branched, aseptate, 1.0–3.0 $\mu$  diameter; (c) broad hyphae observed in some isolates, with walls slightly thickened, brown, branched, frequently septate, up to 9.0 $\mu$  diameter; (d) cuticular cells arising as irregular swellings on hyphae, at first hyaline with contents staining in phloxine, then dark brown and apparently empty, closely packed together to form a pseudoparenchymatous layer, observed in most isolates. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) some broad hyphae as in (c) above.

**TYPE OF ROT**: white rot of broad-leaved trees.



**DESCRIPTIONS OF CULTURAL CHARACTERS:** Campbell (42), Cartwright and Findlay (55, 56), Davidson, Campbell, and Blaisdell (64), Fritz (74), Hopp (87), Humphrey and Siggers (92), Noecker (118), Verrall (149).

Verrall (149) studied a large number of cultures of *Fomes igniarius* from various hosts and found that they fell into three groups, one consisting of isolates from *Populus* spp., one from *Betula* spp., and one from miscellaneous hosts. The form on *Populus* spp. was segregated by Campbell (42) under the name *Fomes igniarius* var. *populinus* and this practice has been followed in the present study. The isolates from hosts other than *Populus* spp. showed considerable variation, but no more than that exhibited by individual isolates at different times. Hence no correlation of growth type with host seemed possible, and all the cultures were included under *Fomes igniarius*. In preparing a description of the species, an attempt has been made to include this range in growth characters. Separation from other species having the same key pattern must be on the basis of color and topography, as noted in the descriptive key.

***Fomes igniarius* var. *laevigatus* (Fries) Overh.**

**KEY PATTERN:** 1 2 1 2 (5,9) 2 2 2 (1,2) 2 1

**CULTURES EXAMINED:**

CANADA.—Ontario: Timagami, on *Betula papyrifera*, F789, on *Betula* sp., F902. British Columbia: Lumby, on *Betula* sp., 9008. UNITED STATES.—Pennsylvania: Detweiler Run, F1956.

**CULTURAL CHARACTERS:** (Pl. III, Fig. 5; Pl. IV, Figs. 20 to 22).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, appressed, hyaline to white, with hyphae well separated. Mat with white border, changing through "cream-buff" (3.0YR8.3/4.5), "chamois" (2.0Y 7.5/5.8), "honey yellow" (2.0Y6.7/6.2), and "clay color" (10.0YR5.8/6.0) to "tawny-olive" (8.0YR4.8/5.8) (one to three weeks), to "sandal brown" (7.0YR5.0/5.5) (four weeks), with little subsequent change in color, slightly raised, cottony in newest growth, then woolly to tufted felty, sometimes interrupted by zones in which aerial mycelium consists of thin white bloom over dark crustose areas, these later being overgrown with felty mycelium. Reverse unchanged at first, then "ochraceous-tawny" (6.0YR4.9/6.3), "cinnamon-brown" (5.0YR 3.0/3.0), and "mummy brown" (7.5YR2.5/2.3) in patches, finally (three to six weeks) all "liver brown" (1.0YR2.3/4.0) and "carob brown" (2.0YR3.1/2.3). Mat peels from agar readily. Odor of wintergreen strong. On gallic and tannic acid agars diffusion zones moderately strong, diameter on gallic acid agar 1.0–2.5 cm., on tannic acid agar no growth to 2.0 cm. diameter.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, with simple septa, frequently branched, 2.0–6.0  $\mu$  diameter. *Aerial mycelium:* (dark brown in potassium hydroxide) (a) hyphae as in advancing zone plentiful; (b) fiber hyphae with thick walls, usually dark brown, rarely branched, occasionally septate, 1.5–3.0  $\mu$  diameter; (c) cuticular cells arising as irregular swellings on hyphae, hyaline with contents staining in phloxine, then dark brown, apparently empty, occurring in loosely arranged groups on aerial mycelium or, more frequently, closely packed together to form a pseudoparenchymatous layer. *Submerged mycelium:* hyphae as in advancing zone.

**TYPE OF ROT:** white rot of broad-leaved trees; canker of *Betula lutea* (Campbell and Davidson (46)).

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Campbell (42), Davidson, Campbell, and Blaisdell (64).

The rapid to moderately rapid growth, and consistently strong odor of wintergreen should be sufficient to separate cultures of this species from others with which they might be confused.

**Fomes igniarius** var. **populinus** (Neuman) Campbell

KEY PATTERN: 1 2 1 2 (5,9) 2 2 2 4 2 1

CULTURES EXAMINED:

CANADA.—Ontario: Petawawa, on *Populus grandidentata*, F7976, on *P. tremuloides*, F7973.

CULTURAL CHARACTERS: (Pl. III, Fig. 6; Pl. IV, Figs. 23 to 25).

GROWTH CHARACTERS.—Growth very slow, radius 4.2–8.5 cm. in six weeks. Advancing zone even at first, becoming bayed after two to four weeks, white, raised aerial mycelium extending to limit of growth. Mat with white border, then "colonial buff" (6.0Y8.5/5.5), "chamois" (2.0Y7.5/5.8), "old gold" (3.0Y5.8/5.5) (one week), becoming "honey yellow" (2.0Y6.7/6.2), "Isabella color" (1.0Y5.5/4.3), "snuff brown" (7.0YR3.9/3.5), and "Saccardo's umber" (9.0YR3.8/3.5) (two to five weeks), to "buckthorn brown" (8.0YR 4.8/6.5) and "cinnamon-brown" (5.0YR3.0/3.0) (six weeks), raised, thick woolly to plush-like, frequently grown to top of Petri dish near inoculum, and sloping gradually to edge, with characteristic grooves radiating from inoculum to marginal bays, some isolates occasionally producing crustose areas, dark brown with thin white bloom over surface. Reverse unchanged in color, with conspicuous zones and radiating lines, except below crustose areas, where agar is "Mars brown" (4.5YR2.8/3.0). Mat peels from agar readily, leaving white bloom. Odor of wintergreen strong. On gallic and tannic acid agars diffusion zones weak to moderately strong, no growth on either medium.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with inconspicuous simple septa, frequently branched, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (turns brown in potassium hydroxide) (a) hyphae as in advancing zone; (b) fiber hyphae buffy brown to dark brown, walls thick and lumina narrow except at tips, rarely branched, aseptate, 1.5–4.5  $\mu$  diameter, curving, interwoven to form mat; (c) cuticular cells arising as swellings on hyphae or as inflated hyphal tips, at first hyaline with contents stained in phloxine, then dark brown, apparently empty, firmly packed together to form pseudoparenchymatous layer. *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white rot of *Populus* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Campbell (42), Davidson, Campbell, and Blaisdell (64), Fritz (74), Hopp (87), Verrall (149).

The slow-growing thick woolly mat over agar that remains unchanged in color or becomes dark only below isolated crustose areas, the strong odor of wintergreen, and the occurrence only on *Populus* spp. make *Fomes igniarius* var. *populinus* distinct from all other species treated in this study.

**Fomes nigrolimitatus** (Romell) Egel.

KEY PATTERN: 2 2 1 2 (3,9) 2 2 2 4 2 1

CULTURES EXAMINED:

CANADA.—British Columbia: Vancouver, on *Picea* sp., 9159, 10085.

ALASKA.—On *P. sitchensis*, 11686.

CULTURAL CHARACTERS: (Pl. III, Fig. 7; Pl. IV, Figs. 26 to 29).

GROWTH CHARACTERS.—Growth very slow, radius 4.0–6.0 cm. in six weeks. Advancing zone even for two to three weeks, then deeply bayed, with slightly raised aerial mycelium extending to limit of growth, forming white border, broad at first, becoming narrower after three to four weeks. Mat "chamois" (2.0Y7.5/5.8), "yellow ochre" (10.0YR6.8/9.0), and "raw sienna" (8.0YR5.3/8.5) (two weeks) to "ochraceous-tawny" (6.0YR4.9/6.3),

"buckthorn brown" (8.0YR4.8/6.5), and "cinnamon-brown" (5.0YR3.0/3.0) near inoculum (four weeks), slightly raised, loosely arranged, cottony-woolly, thin enough to allow darker color of agar to show through, becoming duller in color—"deep brownish drab" (2.0YR3.7/1.5), "buckthorn brown" (8.0YR4.8/6.5), "cinnamon-brown" (5.0YR3.0/3.0), and "Prout's brown" (5.5YR2.8/3.2) (six weeks), collapsed, thin felty. Reverse colored after one week, sometimes green in narrow zone, then "ochraceous-tawny" (6.0YR4.9/6.3), "hazel" (3.0YR3.8/5.0), and "bister" (4.5YR3.0/3.0), the diffusion zone occasionally extending beyond mat, in which case growth is retarded. Odor none or indistinct. On gallic and tannic acid agars diffusion zones strong, trace of growth on both media.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, branched, with simple septa, 1.5–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) hyphae with thin walls, pale brown, frequently septate and branched, often collapsed and distorted, 6.0–12.0  $\mu$  diameter; (c) hyphae with dark brown walls, frequently septate and branched, the branches often lying parallel to parent hyphae to form small strands, 1.5–3.0  $\mu$  diameter; (d) bulbils numerous, formed on narrow brown hyphae, apparently by one or more narrow branches coiling tightly about parent hypha. *Submerged mycelium*: (a) hyphae and (b) bulbils as described above; (c) crystals octahedral, numerous.

**TYPE OF ROT**: white pocket rot of coniferous trees in Western Canada and United States.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Humphrey and Siggers (92).

If, in cultures of *Fomes nigrolimitatus*, bulbils are seen, recognized as such, and included in the key pattern, then the key pattern for this species is unlike that for any other species, and the culture is readily identified. To meet the possibility of bulbils being overlooked in examining cultures, the species is included with those having no "special structures", and falls with *Polyporus dryadeus* and *Poria tsugina*. The restriction of *Fomes nigrolimitatus* to western conifers, the occurrence of bulbils in culture, and the color and topography of its mat, should be sufficient to separate it from these species.

**Fomes officinalis** (Vill. ex Fries) Faull

**KEY PATTERN**: 2 1 2 1 9 1 (1,2) 2 4 2 2

**CULTURES EXAMINED**:

CANADA.—British Columbia: Cowichan, on *Pseudotsuga taxifolia*, 8214; Merritt, on *Pinus ponderosa*, F1309; Queen Charlotte Islands, on *Picea sitchensis*, 10734; Vancouver, on log, 9503. UNITED STATES.—Oregon: Oregon Caves, on *Abies* sp., F1276. Washington: on *Larix occidentalis*, F7196.

**CULTURAL CHARACTERS**: (Pl. III, Fig. 8; Pl. IV, Figs. 30 to 33).

**GROWTH CHARACTERS.**—Growth very slow, radius 4.0–7.0 cm. in six weeks. Advancing zone regular to bayed, in some isolates with raised aerial mycelium to limit of growth, in others with appressed hyaline growth preceding the raised mat. Mat white, compact for two to three weeks, cottony to velvety, uniform, then more loosely arranged, cottony to woolly, more or less patchy, with some areas in which aerial mycelium is scanty and appressed, other areas in which it is raised, frequently appearing farinaceous over cottony mycelium. Reverse unchanged for two to three weeks, then slightly yellowish to "honey yellow" (2.0Y6.7/6.2). Odor fragrant, fruity. On gallic and tannic acid agars no diffusion zones, trace of growth to 2.0 cm. diameter on gallic acid agar, no growth or only a trace on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, thin-walled, with conspicuous clamp connections, frequently branched, 3.0–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) conidia numerous, borne singly at the tips of branches that are usually narrower than the main hyphae, subglobose to ovoid, thin-walled, 4.5–7.5  $\times$  3.0–4.5  $\mu$ ; (c) chlamydospores numerous, terminal and intercalary, with fairly thick walls and contents staining deeply in phloxine, 10.5–15.0  $\times$  6.0–9.0  $\mu$ . *Submerged mycelium*: (a) hyphae and (b) chlamydospores as described above; (c) crystals numerous, octahedral.

**TYPE OF ROT:** brown cubical rot of coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Campbell (42), Cartwright and Findlay (54, 56), Davidson, Campbell, and Blaisdell (64), Faull (70), Humphrey and Siggers (92).

Authors who have described the cultural characters of this species have considered all the spores to be chlamydospores. It would appear, however, that the spores described above as conidia differ from the chlamydospores in their occurrence on aerial mycelium exclusively, their formation at the tips of specialized branches, their smaller size, and their thin walls. When the spores are considered as conidia and chlamydospores, the resulting key pattern does not coincide with those for any other species; if they are interpreted as being all chlamydospores, then the key pattern coincides with that for *Poria asiatica*, the chlamydospores of which are usually rough-walled, and all of large size. Cultures of *Fomes officinalis* resemble closely the cultures of *Poria carbonica*, but the characteristic special hyphae of the latter species, as well as its more rapid rate of growth, make separation of the two species possible.

**Fomes ohiensis** (Berk.) Murr.

**KEY PATTERN:** 1 1 1 1 9 2 2 2 4 2 2

**CULTURES EXAMINED:**

UNITED STATES.—Locality and host unknown, F8005.

**CULTURAL CHARACTERS:** (Pl. III, Fig. 9; Pl. IV, Fig. 34).

**GROWTH CHARACTERS.**—Growth very slow, radius 4.6–5.8 cm. in six weeks. Advancing zone even, hyaline, appressed. Mat white, very thin, appressed, zonate, farinaceous with "dots" of more compact mycelium, forming a tough film on surface of agar. Reverse unchanged. Odor yeasty. On gallic and tannic acid agars diffusion zones moderately strong to strong, no growth on gallic acid agar, trace on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, frequently branched, 2.2–3.0(–4.5) $\mu$  diameter. Hyphae at surface and within agar all like those of advancing zone.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Campbell (42), Davidson, Campbell, and Blaisdell (64).

This description of *Fomes ohiensis* is based on the only culture of the species available, but it agrees closely with the description given by Campbell (42), and so has been included. In the key, *F. ohiensis* coincides with only one other species, *Stereum Murrarii*, their slow rate of growth separating them from all other species with similar characters. Macroscopically, cultures of *Fomes ohiensis* and *Stereum Murrarii* are very different, the former being appressed, farinaceous, with dots of more compact mycelium, the latter being slightly raised, furry or velvety, all compactly arranged and opaque.

**Fomes Pini** (Thore) Lloyd

KEY PATTERN: (1,2) 2 1 2 (2,7) 2 2 2 4 2 1

## CULTURES EXAMINED:

CANADA.—Quebec: Champlain County, on *Picea glauca*, 10267, 10268, on *P. mariana*, 10265, on *Pinus Banksiana*, 10266, 10269, 10306; Gaspé County, on *Picea mariana*, F2366, on *P. rubra*, F955; Montreal, on conifer, 10204. British Columbia: Queen Charlotte Islands, on *P. sitchensis*, 10729, 11725, 11726, 11727, 11728. UNITED STATES.—Virginia: F2162. Host and locality not known, F372, F2163.

CULTURAL CHARACTERS: (Pl. III, Fig. 10; Pl. IV, Figs. 35 to 38).

GROWTH CHARACTERS.—Growth very slow, radius 5.0–8.0 cm. in six weeks. Advancing zone even, white, raised aerial mycelium extending to limit of growth. Mat white to "colonial buff" (6.0Y8.5/5.5), "cream-buff" (3.0Y8.3/4.5), and "chamois" (2.0Y7.5/5.8) (one week), to "primuline yellow" (1.5Y7.5/9.5) and "honey yellow" (2.0Y6.7/6.2) (two weeks), with flecks or patches of "tawny-olive" (8.0YR4.8/5.8), "antique brown" (8.0YR4.2/5.5), or "cinnamon-brown" (5.0YR3.0/3.0) in some isolates (three to six weeks), raised, cottony-woolly to woolly, tufted to form a rough surface, occasionally with sectors or small areas in which aerial mycelium is lacking and colored agar is exposed. Reverse more or less completely colored, "ochraceous-tawny" (6.0YR4.9/6.3), "Sudan brown" (5.5YR3.8/5.5), "Brussels brown" (5.0YR3.5/4.0), and "Prout's brown" (5.5YR2.8/3.2). Odor none. On gallic and tannic acid agars diffusion zones weak to moderately strong, trace of growth to 1.5 cm. diameter on both media.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with simple septa, 2.0–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) hyphae with slightly thicker walls, hyaline to greenish-yellow, with contents hyaline, yellow, or brown, septate, branched, 2.2–6.0  $\mu$  diameter, characteristically with scattered dark brown cells in hyaline or pale hyphae, frequently helioid; (c) expansions on hyphae up to 7.5  $\mu$  diameter, occurring singly or in series, in a terminal or intercalary position, usually with walls brown and thickened so that lumina are visible only in expansions; (d) setae numerous in some isolates, rare or apparently lacking in others, slender, pointed, with walls thick and dark brown, 30.0–67.0  $\times$  4.5–7.5  $\mu$ . *Submerged mycelium*: hyphae as in advancing zone, up to 7.5  $\mu$  diameter.

TYPE OF ROT: red ring rot of coniferous and, rarely, broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Abbott (1), Badcock (3), Campbell (42), Cartwright and Findlay (51, 54, 56), Davidson, Campbell, and Blaisdell (64), Fritz (74), Humphrey and Siggers (92), Owens (125), Percival (126).

*Fomes Pini* occurs frequently among the cultures of wood-decaying fungi received for identification. Recognition of the species is difficult because of the variability of the cultures, which has already been recorded by Owens (125) and others. The occurrence of setae is a useful diagnostic character when present, but they have not been found in all isolates growing in Petri dishes, and they were found only rarely in cultures growing in tubes. Their occurrence in cultures of this species has not been mentioned by other authors. The brown thick-walled hyphae with swellings occurring in series or singly have been observed in all isolates of *F. Pini*, although their appearance in some isolates is delayed until the cultures are six weeks old. These structures constitute a reliable criterion for the separation of *F. Pini* cultures from those of other species.

**Fomes pinicola** (Sw.) Cooke

KEY PATTERN: (1,2) 1 2 1 9 (1,2) 2 2 (2,3) 2 2

## CULTURES EXAMINED:

CANADA.—Quebec: Portage River, on *Picea mariana*, 11594; Gaspé County, F957. Ontario: Minaki, F1268. Manitoba: Victoria Beach, on *Populus balsamifera*, F3249. Saskatchewan: Lake Waskesiu, on conifer, F2380. Alberta: Calgary, on *Populus balsamifera*, F3510. British Columbia: Kaslo, on *Prunus* sp., 8206; Lumby, on *Picea Engelmanni*, 9033; Mt. Arrowsmith, on *Tsuga heterophylla*, 8568; Queen Charlotte Islands, on *Picea sitchensis*, 11142, 11146, 11150, 11151. United States.—Alaska: Hot Springs, on *P. glauca*, F6925. California: Plumas National Forest, on *Abies concolor*, F6624. Maine: Van Buren, on *A. balsamea*, 8234. POLAND.—Pulawy, on *Prunus avium*, F7121; Rabka, on *Picea excelsa*, F7120. JAPAN.—Hokkaido, on *P. yezoensis*, F6895.

CULTURAL CHARACTERS: (Pl. III, Fig. 11; Pl. IV, Figs. 39 to 43).

GROWTH CHARACTERS.—Growth moderately rapid to slow, plates covered in three to six weeks. Advancing zone even, hyaline and appressed in narrow zone, the hyphae appearing coarse and curving by transmitted light. Mat white, at first slightly raised, cottony or cottony-woolly, most isolates remaining unchanged and uniform in appearance, others forming scattered dots of more compact mycelium, especially noticeable by transmitted light, the remainder of the cultures becoming appressed, subfelty or farinaceous around inoculum. Reverse unchanged. Odor none or faintly of apples. On gallic and tannic acid agars no diffusion zones, colonies 1.5–4.0 cm. diameter on gallic acid agar, trace to 3.0 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae nodose-septate, frequently branched at and between septa, 1.5–4.5(–6.0) $\mu$  diameter; (b) chlamydospores numerous in some isolates, rare or lacking in the remainder, usually limited to the advancing zone, especially in mycelium that is grown against wall of Petri dish remote from inoculum, terminal and intercalary, with walls thin or slightly thickened, 6.0–18.0  $\times$  6.0–9.0 $\mu$ , apparently disappearing (probably through germination) from older parts of mat in most isolates. *Aerial mycelium*: (a) hyphae as in advancing zone, frequently with contents that appear oily; (b) fiber hyphae numerous, with walls thick and refractive, lumina narrow or apparently lacking, aseptate, only rarely branched, 1.0–4.5 $\mu$  diameter, closely interwoven; (c) chlamydospores as described above in a few isolates. *Submerged mycelium*: (a) hyphae as in advancing zone, but more frequently branched, up to 9.0 $\mu$  diameter; (b) crystals numerous, octahedral.

TYPE OF ROT: brown cubical rot of coniferous and broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Campbell (42), Cartwright and Findlay (54, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Fritz (74), Humphrey and Siggers (92), Mounce (109), Mounce and Macrae (115).

The variability of cultures of *Fomes pinicola* results in the species appearing in eight places in the key, and necessitates its separation from a number of other species with identical key patterns. Descriptive keys have been inserted to facilitate this separation but the lack of distinctive characters by cultures of *F. pinicola* makes this difficult. However, once one has become familiar with the appearance of cultures of *F. pinicola*, they are readily recognized, more by this lack of distinctive markings than by any characteristic that can be included in a key or description.

**Fomes pomaceus** (Pers.) LloydSee *Fomes fulvus*.**Fomes rimosus** Berk.

KEY PATTERN: 1 2 1 2 9 2 2 2 (2,3) 2 1

## CULTURES EXAMINED:

UNITED STATES.—Maryland: Bethesda, on *Robinia pseudoacacia*, F1690. New York: Spafford, on *R. pseudoacacia*, F1305. North Carolina: Asheville, on *R. pseudoacacia*, F2164. Locality and host not known, F2165.

CULTURAL CHARACTERS: (Pl. III, Fig. 12; Pl. IV, Figs. 44 and 45).

GROWTH CHARACTERS.—Growth moderately rapid to slow, plates covered in four to six weeks. Advancing zone even, hyaline to white, submerged or sparsely downy. Mat with white border, changing abruptly to "clay color" (10.0YR5.8/6.0), "yellow ochre" (10.0YR 6.8/9.0), or "buckthorn brown" (8.0YR4.8/6.5) (one week), later with greater range of colors, from white through "colonial buff" (6.0Y8.5/5.5), "cream-buff" (3.0Y8.3/4.5), "chamois" (2.0Y7.5/5.8), "tawny-olive" (8.0YR4.8/5.8), to "old gold" (3.0Y5.8/5.5) and "mustard yellow" (2.0Y7.8/7.5) (four to six weeks), the brighter colors frequently assumed by the older cultures being noteworthy, at first appressed, downy to cottony, becoming cottony-floccose, finally felty and tufted. Reverse unchanged below newest growth, then "ochraceous-tawny" (6.0YR4.9/6.3), "cinnamon-brown" (5.0YR3.0/3.0), and "Prout's brown" (5.5YR2.8/3.2). Odor none. On gallic and tannic acid agars diffusion zones weak to moderately strong, trace of growth on both media.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with simple septa, branched, 3.0–7.5 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, becoming yellow and brown; (b) fiber hyphae with walls slightly thickened, occasionally pale yellow, more frequently dark brown, branched, occasionally septate, 2.0–3.0 $\mu$  diameter. *Submerged mycelium*: (a) hyphae as in advancing zone, frequently branched; (b) crystals numerous, octahedral.

TYPE OF ROT: spongy yellow heart rot of living *Robinia pseudoacacia*.

DESCRIPTIONS OF CULTURAL CHARACTERS: Campbell (42), Davidson, Campbell, and Blaisdell (64), Humphrey and Siggers (92), Long and Harsch (98).

*Fomes rimosus* is usually considered to be specific to *Robinia pseudoacacia*, and this host relationship, along with the occurrence of unusually broad hyphae in the cultures, should aid in the separation of this species from the numerous other species that have identical key patterns.

**Fomes roseus** (Alb. and Schw. ex Fries) Cooke

KEY PATTERN: (1,2) 1 (1,2) 1 9 2 2 2 2 1 (1,2)

## CULTURES EXAMINED:

CANADA.—Quebec: Gaspé County, on *Picea glauca*, F2355. Ontario: Timagami, on *P. glauca*, F1449. British Columbia: Vancouver, on *Pseudotsuga taxifolia*, F2374. UNITED STATES.—Host and locality not known, F2929.

CULTURAL CHARACTERS: (Pl. III, Fig. 13; Pl. IV, Figs. 46 to 49).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three weeks. Advancing zone even, hyaline and appressed so that it is difficult to see limit of growth. Mat white to "tulleul buff" (9.0YR8.7/1.5), "shell pink" (2.0YR8.4/3.7), "light pinkish cinnamon" (7.0YR7.5/4.5), "vinaceous-buff" (7.0YR7.0/3.5), and "vinaceous-fawn" (4.5YR6.7/3.5) around inoculum after three to four weeks, downy or farinaceous in some isolates, becoming more compact felty after three to four weeks and forming a granular to irregularly pored fruiting surface. Reverse unchanged or with scattered areas "hazel" (3.0YR3.8/5.0) to "Prout's brown" (5.5YR2.8/3.2) after five to six weeks. Odor slightly fruity. On gallic and tannic acid agars diffusion zones lacking or very weak, diameter 2.0–2.5 cm. on gallic acid agar, trace to 1.5 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, frequently branched, 1.5–4.5(–6.0) $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae rare to numerous, with walls thick and refractive, lumina narrow or apparently lacking, sparingly branched, apparently aseptate, 2.0–3.0 $\mu$  diameter. *Fruit body*: (a) nodose-septate and (b) fiber hyphae as above; (c) basidia 4.5 $\mu$  diameter, bearing four spores; (d) basidiospores hyaline, even, elongate-ellipsoid, slightly curved, 4.5–6.0  $\times$  1.5–2.7 $\mu$ . *Submerged mycelium*: (a) hyphae as in advancing zone; (b) crystals numerous, in irregular clumps.

**TYPE OF ROT**: brown cubical rot of coniferous and, rarely, broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Campbell (42), Cartwright and Findlay (54), Davidson, Campbell, and Blaisdell (64), Davidson, Lombard, and Hirt (68), Fritz (74), Mounce and Macrae (114), Snell (138), Snell, Hutchinson, and Newton (140).

In the key no code number is allotted to a color change from white to pink and vinaceous, and cultures exhibiting these colors fall with cultures that remain white. Thus the key patterns for *Fomes roseus* may coincide with those for *Pleurotus ostreatus*, *Polyporus hirsutus*, *Schizophyllum commune*, and others, from all of which separation can usually be made on the basis of color. Cultures of *Fomes roseus* and *F. subroseus* are similar and have been separated by Snell, Hutchinson, and Newton (140), and Campbell (42) on the basis of their different rates of growth at certain temperatures. In the present study, in the four cultures of *F. roseus* examined, no chlamydospores were observed, while they occurred in all eight cultures of *F. subroseus* studied. Two cultures filed in the stock culture collection under *F. roseus* were found to have chlamydospores. On carrying out interfertility tests between these cultures and authentic cultures of *F. roseus* and *F. subroseus* it was found that they were interfertile with *F. subroseus* and therefore belonged to that species and not to *F. roseus*. This provided additional evidence, but more is required to establish definitely that chlamydospores are regularly present in *F. subroseus*, absent in *F. roseus*.

### ***Fomes scutellatus* (Schw.) Cooke**

**KEY PATTERN**: 1 2 1 1 9 2 2 2 (2,3) (1,2) 2

#### **CULTURES EXAMINED:**

**CANADA**.—Ontario: Chalk River, on *Alnus incana*, F3255; Ottawa, on *A. incana*, F3444.

**CULTURAL CHARACTERS**: (Pl. III, Fig. 14; Pl. IV, Figs. 50 to 52).

**GROWTH CHARACTERS**.—Growth moderately rapid to slow, plates covered in three to six weeks. *Advancing zone* even, hyaline, appressed or with slightly raised aerial mycelium to limit of growth. Mat white at first, becoming "cream-buff" (3.0Y8.3/4.5) to "cinnamon-brown" (5.0YR3.0/3.0) in isolated areas after three to four weeks, the first growth over the inoculum cottony to woolly, appressed over agar, becoming slightly raised, floccose to cottony-woolly, lacunose, forming a tough, coherent mat even where thin, after three to four weeks with scattered loose-cottony to compact-felty balls of mycelium, some of which develop granular fruiting surfaces after five to six weeks. Reverse unchanged. Odor of almonds. On gallic and tannic acid agars diffusion zones weak to moderately strong, no growth on gallic acid agar, no growth or only a trace on tannic acid agar.

**HYPHAL CHARACTERS**.—*Advancing zone*: hyphae hyaline with conspicuous clamp connections, frequently branched at and between septa, 1.5–6.0 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, becoming greenish-yellow in colored areas; (b) fiber hyphae



abundant, thick-walled with narrow lumina, apparently aseptate, frequently branched, the branch forming a right angle with the parent hypha, curving and firmly interwoven, 1.5–3.0  $\mu$  diameter; (c) basidiospores (from spore deposit) cylindric, 7.5–8.0  $\times$  2.2–3.0  $\mu$ . *Submerged mycelium*: hyphae hyaline, thin-walled, nodose-septate, with many short irregular branches and projections, 2.2–4.5  $\mu$  diameter; (c) crystals numerous, needlelike.

**TYPE OF ROT:** white rot of broad-leaved trees, usually *Alnus* spp.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Campbell (42), Davidson, Campbell, and Blaisdell (64).

Faster-growing cultures that fruit and all slow-growing cultures of *Fomes scutellatus* have key patterns that fall alone in the key, but cultures that cover the plates in three to four weeks and fail to fruit have a key pattern that coincides with those for *Collybia radicata* and *Daedalea confragosa*. Under each of these species mention is made of distinguishing characters, of which the preference of *Fomes scutellatus* for *Alnus* spp. is most helpful.

**Fomes subroseus** (Weir) Overh.

**KEY PATTERN:** (1,2) 1 (1,2) 1 9 1 2 2 2 1 2

**CULTURES EXAMINED:**

CANADA.—Quebec: Champlain County, on *Picea mariana*, 10278. Ontario: Keewatin, on conifer, F2392. British Columbia: Saanichton, on *Pseudotsuga taxifolia*, 8203, on conifer, 8184. UNITED STATES.—New York: Oswego County, on conifer, F1300. Oregon: Benton County, on *Pseudotsuga taxifolia*, F1639. Host and locality not known, F1000.

**CULTURAL CHARACTERS:** (Pl. III, Fig. 15; Pl. IV, Figs. 53 to 58).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, hyaline and appressed in zone 0.5–1.0 cm. broad. Mat white to "shell pink" (2.0YR8.4/3.7) (one week), "pale vinaceous-pink" (1.0YR7.8/3.3), and "vinaceous-pink" (2.0YR7.1/4.5) (two weeks), to "vinaceous-fawn" (4.5YR6.7/3.5) (four weeks), at first downy, becoming slightly raised, cottony (two weeks), to floccose-cottony and pitted (four weeks), with pored areas over inoculum after one to two weeks and covering most of surface after six weeks, waxy, the dissepiments thick and pores relatively small. Reverse unchanged. Odor sweet. On gallic and tannic acid agars diffusion zones lacking or very weak, diameter 1.5–3.0 cm. on gallic acid agar, trace to 1.5 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, occasionally up to 6.0  $\mu$  diameter, with walls irregularly thickened; (b) fiber hyphae numerous, with walls thick and lumina narrow or apparently lacking, occasionally branched, aseptate, 1.5–4.0  $\mu$  diameter; (c) chlamydospores rare, intercalary or terminal, walls thin, ovoid to elongate, 10.5–24.0  $\times$  6.0–7.5  $\mu$ . *Fruit body*: (a) basidia 4.5–6.0  $\mu$  diameter, bearing four spores; (b) basidiospores hyaline, even, allantoid, 6.0–8.0  $\times$  1.5–2.0  $\mu$ . *Submerged mycelium*: (a) nodose-septate hyphae and (b) chlamydospores as described above; (c) crystals numerous, irregular, frequently in large aggregates.

**TYPE OF ROT:** brown cubical rot of coniferous and, rarely, broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Campbell (42), Cartwright and Findlay (54, 56), Davidson, Campbell, and Blaisdell (64), Humphrey and Siggers (92), Mounce and Macrae (114), Snell, Hutchinson, and Newton (140).

*Fomes subroseus*, having two variable characters, falls with four groups of species in the numerical key. The distinctive "shell pink" to "vinaceous-pink" color of the mat is characteristic, and not to be confused with the color

of any other species having the same key pattern. Its similarity to *F. roseus* is discussed under that species, with evidence that chlamydospores occur only in *F. subroseus* and so provide a means of separating the two species.

**Ganoderma applanatum** (Pers.) Pat.

KEY PATTERN: (1,2) (1,2) 1 1 5 2 2 2 (2,4) 2 2

CULTURES EXAMINED:

CANADA.—Quebec: Eardley, on *Abies balsamea*, F7524; Ste. Anne de la Pocatière, on *Pyrus* sp., F6914. Ontario: Ottawa, on *Quercus* sp., 9295. British Columbia: Saanichton, on *Pseudotsuga taxifolia*, 8180. UNITED STATES.—Locality and host not known, F2019, F2026, F2159. JAPAN.—Kyoto, F1342.

CULTURAL CHARACTERS: (Pl. V, Fig. 1; Pl. VI, Figs. 1 to 4).

GROWTH CHARACTERS.—Growth moderately rapid in some isolates, plates covered in three weeks, to very slow in others, plates covered in six or more weeks. Advancing zone even, appressed or with slightly raised downy mycelium to limit of growth. Mat white at first and remaining so or becoming "tilleul buff" (9.0YR8.7/1.5) to "avellaneous" (8.0YR 6.2/3.5), "cream buff" (3.0Y8.3/4.5) to "Isabella color" (1.0Y5.5/4.3), or "olive-buff" (4.0Y7.5/3.0) to "dark olive-buff" (4.0Y6.5/4.5) after two to four weeks, the newest growth appressed, translucent, cottony or farinaceous, later opaque, compact, felty or pellicular in scattered areas or over whole surface, the color occurring in these compact areas, frequently wrinkled. Reverse unchanged or "olive-ocher" (3.0Y6.8/6.5) to "honey yellow" (2.0Y 6.7/6.2) under colored areas. Odor none. On gallic and tannic acid agars diffusion zones weak to strong, no growth to 1.0 cm. diameter on gallic acid agar, trace to 1.5 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, branched, the branches usually occurring at the septa, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae very numerous, with walls thick and refractive, lumina narrow or apparently lacking, aseptate, frequently branched, 1.0–2.0(–4.5)  $\mu$  diameter, closely interwoven; (c) cuticular cells (formed at surface of agar) appearing first as swellings on nodose-septate hyphae, with contents staining in phloxine, later empty, with walls slightly thickened, compactly arranged to form pseudoparenchymatous layer; (d) staghorn hyphae present in some isolates. *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white mottled rot of broad-leaved or, rarely, coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Campbell (42), Cartwright and Findlay (51, 52, 53, 55, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Fritz (74), Hopp (88), Humphrey and Siggers (92), White (151).

Coleman (59), having made a careful study of the spore wall in several species of *Ganoderma*, wrote "It would seem to be a character of much greater importance from a systematic standpoint than many of those at present being used in the classification of the Polyporaceae", and "I consider that all forms showing the spore characteristics described should be brought together under the genus *Ganoderma* Karst". Those taxonomists who concur in this opinion follow Patouillard in including *Ganoderma applanatum* in this genus, while others who consider the structure of the upper surface of the fruit body as a more important diagnostic character in delimiting the genus, put the species under *Fomes applanatus*. In the present study, its cultural characters were found to be so similar to those of the species of *Ganoderma* examined as to indicate relationship, and to warrant its inclusion in that genus. In fact, its

separation from other species of *Ganoderma* with which it coincides in the key is exceedingly difficult, and information such as that *G. oregonense* is limited to western conifers and that *G. lobatum* is rare and will probably not be encountered in Canada, must be considered in addition to cultural characters in making identifications.

### ***Ganoderma lobatum* (Schw.) Lowe**

KEY PATTERN: 1 2 1 1 5 2 2 2 2 2

#### **CULTURES EXAMINED:**

UNITED STATES.—Indiana: on old stump, F8008. Louisiana: on *Acer* sp., F8007. Maryland: on *Quercus* sp., F8006.

CULTURAL CHARACTERS: (Pl. V, Fig. 2; Pl. VI, Figs. 5 to 8).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone hyaline to white, even, fimbriate. Mat distinctly two-zoned: outer zone 1–2 cm. broad, white, slightly raised, short cottony with radiating growth lines; inner zone "cream-buff" (3.0Y8.3/4.5) (one week) to "chamois" (2.0Y7.5/5.8) and "avellaneous" (8.0YR6.2/3.5), appressed, farinaceous to felty, becoming cartilaginous and furrowed, this type of growth finally extending over whole surface. Reverse unchanged to "chamois" (2.0Y7.5/5.8) and "honey yellow" (2.0Y6.7/6.2) below central part, conspicuously veined. No odor. On gallic and tannic acid agars diffusion zones weak to moderately strong, no growth to 2.0 cm. diameter on gallic acid agar, trace to 3.0 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, thin-walled, nodose-septate, frequently branched, 2.2–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae numerous, hyaline to buffy brown, with walls thick and lumina narrow or apparently lacking, frequently branched, 2.0–3.0  $\mu$  diameter; (c) cuticular cells arising as globose to irregular swellings on nodose-septate hyphae, hyaline to buffy brown, closely packed together and interwoven with fiber hyphae to produce the tough felty pellicle of the inner zone; (d) staghorn branched hyphae numerous in some isolates, rare in others, produced as branches from nodose-septate hyphae. *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white rot of broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Campbell (42), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67).

Lowe (99), on the basis of the morphological characters of the fruit body, transferred this species from *Fomes* to *Ganoderma* and this disposition of it is corroborated by cultural studies, which have shown cultures of *G. lobatum* to be similar to those of the other species of *Ganoderma* examined. Cultures of *G. applanatum* and *G. lobatum* are almost indistinguishable. Fruit bodies of *G. lobatum* are found only rarely and no collections have been recorded from Canada except from the southernmost part of Ontario, while *G. applanatum* has a wide host and geographic range. Hence there is much greater likelihood of encountering *G. applanatum* than *G. lobatum* in cultures from rots.

### ***Ganoderma lucidum* (Leyss. ex Fries) Karst.**

KEY PATTERN: 1 (1,2) 1 1 5 1 2 2 1 2 3

#### **CULTURES EXAMINED:**

CANADA.—Ontario: Ottawa, on *Gleditsia macrantha*, 10222. UNITED STATES.—On *Quercus* sp., F2040. Host and locality not known, F3055.

# CULTURAL CHARACTERS: (Pl. V, Fig. 3; Pl. VI, Figs. 9 to 13).

**GROWTH CHARACTERS.**—Growth rapid, plates covered in two weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white and remaining so or becoming "wood brown" (7.0YR5.7/4.0) (two weeks), with color subsequently masked by overgrowth of whitish 'bloom', at first slightly raised, cottony, then appressed, felty to pellicular with farinaceous surface, traversed by deep wrinkles or grooves. Reverse unchanged for two or three weeks, then with scattered patches of "honey yellow" (2.0Y6.7/6.2) to "buckthorn brown" (8.0YR4.8/6.5), finally bleached, with wrinkles prominent, producing a veined appearance. No odor. On gallic acid agar diffusion zone weak to strong, no growth or diameter up to 4.0 cm.; on tannic acid agar diffusion zone moderately strong to strong, diameter 2.0-4.0 cm.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 2.2-4.5(-6.0)  $\mu$  diameter. *Aerial mycelium* (thin tough skin that peels from agar): (a) hyphae as in advancing zone, with frequent branches and numerous small projections; (b) fiber hyphae very numerous, with walls thick and refractive, lumina narrow or apparently lacking, except in main hyphae, frequently branched, the ends long, slender, curving and interwoven, 1.0-3.0  $\mu$  diameter; (c) cuticular cells thin-walled, produced by inflation of nodose-septate hyphae, at first with contents staining in phloxine, then empty, closely compacted and interwoven with fiber hyphae and staghorn hyphae to form pseudoparenchymatous layer, which may remain hyaline or become brown; (d) chlamydospores very numerous, walls slightly thickened, terminal and intercalary, broadly ovoid to elongate, 12.0-21.0  $\times$  7.5-10.5  $\mu$ ; (e) staghorn branched hyphae with minute branches in all planes very numerous in 10222, not observed in other isolates. *Submerged mycelium:* (a) nodose-septate hyphae and (b) chlamydospores as described above.

**TYPE OF ROT:** soft spongy white rot of broad-leaved trees (see below).

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64) (under *Polyporus lucidus*), Davidson, Campbell, and Vaughn (67) (under *P. lucidus*), Humphrey and Siggers (92).

As a result of a comparison of European collections of *Polyporus lucidus*, which occurs commonly on broad-leaved trees and more rarely on coniferous trees in Europe, with American collections of *Ganoderma Tsugae* from coniferous trees, usually *Tsuga* spp., Atkinson (2) concluded that they should be brought together in one species, *Ganoderma pseudoboletum*. Haddow (79) made a critical study of European and American collections of *G. lucidum* and American collections of *G. Tsugae* and concluded that there were no constant morphological differences and that host specificity was not a valid basis for separation of the species. Therefore he agreed with Atkinson in placing the two under one species, for which he used the accepted name, *Ganoderma lucidum*. Boyce (35), Lowe (99), and others have followed this practice, but Overholts (119) has retained *G. (Polyporus) Tsugae* in specific rank. In the present study the three cultures of *G. lucidum* isolated from fruit bodies on broad-leaved trees were distinguishable from the four cultures of *G. Tsugae* from *Tsuga* spp. on the basis of chlamydospore production, which was prolific in all the cultures of *G. lucidum* and completely lacking in those of *G. Tsugae*. Because of this difference, the species have been treated separately, but this does not constitute an argument for their separation in taxonomic studies, since the number of isolates in each species is too small to provide convincing evidence. Interfertility tests are required to establish conclusively whether one or two species are involved.

*Ganoderma lucidum* is unique among the *Ganoderma* species studied in having chlamydospores and therefore its key pattern does not coincide with that for any of the other species.

**Ganoderma oregonense** Murr.

KEY PATTERN: 2 (1,2) 1 1 5 2 2 2 2 2 (1,2)

## CULTURES EXAMINED:

CANADA.—British Columbia: Little Qualicum River, on *Abies grandis*, F8240; Vancouver, on *Tsuga heterophylla*, F572; on stump, F1012. UNITED STATES.—Oregon: Oregon Caves, on *Abies concolor*, F1274.

CULTURAL CHARACTERS: (Pl. V, Fig. 4; Pl. VI, Figs. 14 to 16).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three weeks. Advancing zone even, hyaline, appressed, with almost no aerial mycelium over outer zone, 1.0–1.5 cm. broad. Mat white at first, with more or less extensive areas of "cream-buff" (3.0Y 8.3/4.5), "chamois" (2.0Y 7.5/5.8), and "old gold" (3.0Y 5.8/5.5) after three to four weeks, appressed, felty with farinaceous surface, wrinkled, the furrows cutting into agar and showing as a reticulate network on reverse. Reverse "honey yellow" (2.0Y 6.7/6.2) to "cinnamon-brown" (5.0YR 3.0/3.0) after three to four weeks. Odor fairly strong, fragrant. On gallic and tannic acid agars diffusion zones moderately strong to strong, no growth or only a trace on gallic acid agar, diameter 1.2–2.0 cm. on tannic acid agar.

HYPHAL CHARACTERS.—Advancing zone: hyphae hyaline, nodose-septate, 1.5–5.0  $\mu$  diameter. Aerial mycelium: (a) hyphae as in advancing zone, becoming broken into short lengths or converted into cuticular cells; (b) cuticular cells elongate to globose, irregular, at first deeply stained in phloxine, later empty, compactly arranged to form a pseudoparenchymatous layer. Submerged mycelium: hyphae as in advancing zone.

TYPE OF ROT: soft spongy white rot of western coniferous trees, especially "western hemlock and balsam fir" (Boyce).

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Davidson, Campbell, and Blaisdell (64).

The lack of fiber hyphae and the permanent color in the agar of cultures of *Ganoderma oregonense* provide reliable means of distinguishing this from other species of *Ganoderma* in culture. In addition, the fact that it is known only on western conifers removes it from the list of species likely to be encountered in other regions and on other hosts.

**Ganoderma Tsugae** Murr.

KEY PATTERN: 2 (1,2) 1 1 5 2 2 2 2 2 (2,3)

## CULTURES EXAMINED:

CANADA.—Quebec: Ile Perrot, on conifer, 10197. Ontario: Chaffey's Locks, on *Tsuga canadensis*, 9346; Matawatchan, on *T. canadensis*, F2275. British Columbia: Vancouver, on *T. heterophylla*, F569.

CULTURAL CHARACTERS: (Pl. V, Fig. 5; Pl. VI, Figs. 17 to 20).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, hyaline, submerged or appressed. Mat white at first and remaining so or developing scattered, more or less extensive, colored areas—"mustard yellow" (2.0Y 7.8/7.5), "primuline yellow" (1.5Y 7.5/9.5), "chamois" (2.0Y 7.5/5.8), "honey yellow" (2.0Y 6.7/6.2), and "buckthorn brown" (8.0YR 4.8/6.5)—the white areas subfelty to felty, with farinaceous surface and scattered dots of more compact mycelium, the colored areas crustlike or lacking aerial mycelium and then with the whole surface glazed, all with conspicuous anastomosing and branching wrinkles and furrows cut into the agar and producing a veined appearance when viewed through the agar. Reverse "honey yellow" (2.0Y 6.7/6.2), sometimes bleached in part. Odor none. On gallic and tannic acid agars diffusion zones moderately strong, no growth on gallic acid agar, diameter 1.5–2.0 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, -nodose-septate, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, frequently empty; (b) fiber hyphae, with walls thick and refractive, lumina narrow or apparently lacking, frequently branched, 1.5–4.0  $\mu$  diameter; (c) cuticular cells, globose or subglobose, at first with contents staining in phloxine, then empty or with yellow or brown contents, closely packed to form a firm skin, cartilaginous in texture; (d) staghorn hyphae present in some isolates, apparently lacking in others. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) crystals numerous, octahedral.

**TYPE OF ROT**: soft spongy white rot of dead coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Davidson, Campbell, and Blaisdell (64).

The cultural characters that have been used in separating *Ganoderma lucidum* and *G. Tsugae* are stated in the discussion under *G. lucidum*. Culturally these two species are sufficiently distinct not to coincide in the key, but *G. Tsugae* has the same key pattern as *G. oregonense* and *G. applanatum*, and it is difficult to separate them on the basis of cultural characters. Distribution and host are helpful, in that *G. Tsugae* is confined to eastern conifers, *G. oregonense* to western coniferous trees, and *G. applanatum*, while widely distributed, occurs more frequently on broad-leaved trees than on conifers.

**Hymenochaete corrugata** (Fries) Lév.

**KEY PATTERN**: (1,2) (1,2) 1 2 9 2 2 2 (1,2) 2 (1,2)

**CULTURES EXAMINED**:

**UNITED STATES.**—Locality and host not known, F2940, F2941.

**CULTURAL CHARACTERS**: (Pl. V, Fig. 6; Pl. VI, Figs. 21 and 22).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone variable, appressed or with raised aerial mycelium extending to limit of growth. Mat white or with scattered areas and lines "chamois" (2.0Y7.5/5.8) and "buckthorn brown" (8.0YR4.8/6.5) appearing after three to four weeks, slightly raised, woolly, at first thin and translucent, later thicker and opaque. Reverse unchanged or with irregular patches of "cinnamon-buff" (9.0YR6.6/5.8), "onion-skin pink" (2.0YR5.8/6.0), "pecan brown" (3.0YR4.7/4.5), to "walnut brown" (2.0YR3.8/3.5) appearing after three or more weeks. Odor sweet. On gallic acid agar diffusion zone strong, diameter up to 1.5 cm.; on tannic acid agar diffusion zone moderately strong, diameter 1.5–6.5 cm.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, with rare simple septa, 3.0–6.0  $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone, but usually narrow, 1.5–2.2  $\mu$  diameter. *Submerged mycelium*: hyphae as in advancing zone; (b) crystals numerous, octahedral.

**TYPE OF ROT**: white rot of broad-leaved or, rarely, coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Davidson, Campbell, and Blaisdell (64).

Only two cultures of *Hymenochaete corrugata* were available for study, and they showed differences in color, rate of growth, and effect on agar. These possible variations, along with the occurrence on both broad-leaved and coniferous trees, necessitates the inclusion of 16 different key patterns for the species. In several instances these coincide with the key patterns for other species but it is hoped that separations can be made by means of the descriptive keys that have been included.

**Hymenochaete tabacina** (Sow. ex Fries) Lév.

KEY PATTERN: (1,2) 2 1 2 6 2 2 2 2 2

## CULTURES EXAMINED:

CANADA.—British Columbia: Saanichton, on *Thuja plicata*, 9252, 9290.

CULTURAL CHARACTERS: (Pl. V, Fig. 7; Pl. VI, Figs. 23 to 26).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three weeks. Advancing zone even, hyaline, slightly raised aerial mycelium extending to limit of growth. Mat white (two weeks), with small areas becoming "Naples yellow" (3.0YR 8/5.0), "fawn color" (4.0YR 5.6/4.2), and "wood brown" (7.0YR 5.7/4.0) (three weeks), these increasing in extent and changing to "vinaceous-fawn" (4.5YR 6.7/3.5) and "brownish drab" (5.0R 5.7/2.0) (four to six weeks), slightly raised, downy to thin woolly to felty. Reverse unchanged or slightly darker below colored areas. Odor none. On gallic and tannic acid agars diffusion zones strong, diameter 1.0–1.5 cm. on gallic acid agar, 2.5–3.5 cm. on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, occasionally branched, with rare simple septa, 3.0–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, frequently branched and anastomosed, 1.5–6.0  $\mu$  diameter; (b) brown hyphae, with walls slightly thickened, branched, with simple septa, 2.2–4.5  $\mu$  diameter; (c) brown hyphae with numerous irregular short branches, compactly interlocked to produce a firm pseudoparenchymatous layer. *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white rot of broad-leaved or, rarely, coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64).

Cultures of *Hymenochaete tabacina* from broad-leaved trees have a key pattern that coincides with that for *Polyporus dryophilus* var. *vulpinus*. Cultures of the latter species have thick cottony mats, all colored except for a white border, and are, therefore, not to be confused with cultures of *Hymenochaete tabacina*.

**Lentinus Kauffmanii** Smith

KEY PATTERN: 2 1 (1,2) 3 9 2 2 2 (2,3) 2 2

## CULTURES EXAMINED:

CANADA.—British Columbia: Prince George, on *Picea* sp., 17180; Queen Charlotte Islands, on *P. sitchensis*, F346, F443, F476, 10722, 10723, 11656, 11657, 11658, 11659, 11660, 11741, 11742, 16057, 16058, 16059. UNITED STATES.—Oregon: on *P. sitchensis*, 11684.

CULTURAL CHARACTERS: (Pl. V, Fig. 8; Pl. VI, Figs. 27 to 29).

GROWTH CHARACTERS.—Growth moderately rapid to slow, plates covered in four to five weeks. Advancing zone even, hyaline and appressed in zone up to 1.0 cm. broad, the mycelium so scanty that limit of growth can be determined only by removing lid of Petri dish and examining surface of colony by reflected light. Mat white at first and remaining so or developing "pinkish buff" (9.0YR 7.3/4.5) to "cinnamon-buff" (9.0YR 6.6/5.8) areas after five to six weeks, appressed, downy to thin woolly, with numerous scattered compact dots or nodules, of which the surface is felty to smooth and skinlike, ranging from 1.0 mm. or less in diameter on young colonies up to 5.0–10.0 mm. on six-weeks-old cultures. Reverse unchanged. Odor strong, disagreeable. On gallic and tannic acid agars diffusion zones lacking or weak, no growth on either medium.

HYPHAL CHARACTERS.—*Advancing zone*: leading hyphae hyaline, thin-walled, with inconspicuous simple septa, frequently branched, the branches soon developing clamp connections, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (a) nodose-septate hyphae as described above; (b) fiber hyphae numerous in older cultures, the walls thick and refractive, lumina narrow or apparently lacking except at tips, occasionally branched, 2.2–3.0  $\mu$  diameter. *Submerged mycelium*: (a) nodose-septate hyphae as described above; (b) crystals numerous, large, octahedral.

TYPE OF ROT: pocket dry rot of *Picea* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Bier and Nobles (30).

Cultures of this species, isolated from either rotted wood or fruit bodies, are remarkably uniform and readily recognizable. If the characteristic hyphae of the advancing zone are observed and included, then the key pattern does not coincide with that for any other species. To date, the fungus has been recorded on spruce along the Pacific Coast from California to the Queen Charlotte Islands, and in the Prince George district of British Columbia.

### ***Lentinus lepideus* Fries**

KEY PATTERN: 2 1 (1,2) 3 9 1 2 2 2 2 2

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Chelsea, on stump, F627. Ontario: Ottawa, on buried wood, F1334. Manitoba: Winnipeg, F3446. UNITED STATES.—Locality and host not known, F371.

CULTURAL CHARACTERS: (Pl. V, Fig. 9; Pl. VI, Figs. 30 to 34).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, submerged and appressed mycelium extending 0.5–1.0 cm. beyond aerial mycelium. Mat white, at first appressed, downy to thin woolly, so thin as to be translucent, becoming slightly raised and more compact woolly in scattered dots or zones, these especially numerous around edge of Petri dish and near inoculum, suggesting immature fruit bodies, "cinnamon-buff" (9.0YR6.6/5.8) to "clay color" (10.0YR5.8/6.0). Reverse unchanged. Odor very strong and disagreeable. On gallic and tannic acid agars diffusion zones lacking or very weak, growth lacking or limited to a trace.

HYPHAL CHARACTERS.—*Advancing zone*: leading hyphae hyaline, with rare inconspicuous simple septa, frequently branched, the branches soon developing clamp connections, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae hyaline, uniform, nodose-septate, 1.5–4.5  $\mu$  diameter; (b) fiber hyphae numerous in older cultures, with walls thick and refractive, aseptate, unbranched, 2.0–3.0  $\mu$  diameter, long curving, tapering to a slender tip; (c) chlamydospores rare to fairly numerous, walls relatively thick and not stained in phloxine, contents deeply stained, globose to broadly ovoid, 10.5–16.5  $\times$  7.5–13.5  $\mu$ . *Submerged mycelium*: (a) nodose-septate hyphae as in aerial mycelium, occasionally swollen up to 10.5  $\mu$  diameter, with walls irregularly thickened and refractive; (b) chlamydospores as described for aerial mycelium; (c) crystals needlelike, often in clumps.

TYPE OF ROT: brown cubical rot of coniferous trees, especially *Pinus* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Cartwright and Findlay (51, 54, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Lombard, and Hirt (68), Snell (138), Walek-Czernecka (150).

Cultures of this species are similar to those of *Lentinus Kauffmanii*, the presence of chlamydospores on cultures of *L. lepideus* and their absence in *L. Kauffmanii* being the only means of separation. As with *L. Kauffmanii*, if the character of the hyphae of the advancing zone is observed and included in the key pattern, then the species does not coincide with any other in the key.

### ***Lentinus tigrinus* Bull. ex Fries**

KEY PATTERN: 1 (1,2) 1 1 9 1 2 2 2 2 2

#### **CULTURES EXAMINED:**

CANADA.—Ontario: Locality and host unknown, F1349. UNITED STATES.—Locality and host not known, F3048.



**CULTURAL CHARACTERS:** (Pl. V, Fig. 10; Pl. VI, Figs. 35 and 36).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three weeks. Advancing zone even, hyaline and appressed, or with aerial mycelium uniform to limit of growth. Mat white and remaining so or becoming "yellow ochre" (10.0YR6.8/9.0), "tawny-olive" (8.0YR4.8/5.8), and "saya brown" (7.0YR5.0/5.5) around inoculum, appressed, at first downy, then fine woolly, with few scattered compact lumps, especially against side of Petri dish in vicinity of inoculum, which may develop into short stipes of abortive fruit bodies (four to six weeks). Reverse unchanged. Odor strong, sharp, and penetrating. On gallic and tannic acid agars diffusion zones strong, diameter trace to 1.5 cm. on gallic acid agar, 1.5–2.0 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 2.2–4.5  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) chlamydospores numerous; terminal and intercalary, thin-walled, 7.5–12.0  $\times$  6.0–9.0  $\mu$ . *Submerged mycelium:* (a) hyphae and (b) chlamydospores as described above.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Cartwright and Findlay (51, 56), Davidson, Campbell, and Blaisdell (64), Snell (139).

The key patterns for *Lentinus tigrinus* coincide with those of several other species, but its thin woolly mat and the common formation of fruit body fundaments should serve to distinguish cultures of *L. tigrinus* from other species.

***Lenzites betulina* L. ex Fries**

**KEY PATTERN:** (1,2) 1 1 1 9 2 2 2 (1,2) 2 2

**CULTURES EXAMINED:**

CANADA.—Quebec: Iberville, on *Fagus grandifolia*, F8021; Montreal, on *Betula* sp., 10199. UNITED STATES.—Host and locality not known, F2171.

**CULTURAL CHARACTERS:** (Pl. V, Fig. 2; Pl. VI, Figs. 37 and 38).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to four weeks. Advancing zone even, in some isolates hyaline and appressed in narrow zone so that it is difficult to see limit of growth, in others with the slightly raised aerial mycelium extending to limit of growth. Mat white, the newest growth slightly raised, floccose-woolly, becoming patchy, with some areas raised, plateaulike, felty-woolly, very tough, the intervening areas appressed, thin felty, all peeling readily from agar, the mycelium frequently grown up sides and down between lid and base of Petri dish. Reverse unchanged. Odor lacking or slight. On gallic and tannic acid agars diffusion zones strong, no growth or only a trace on gallic acid agar, diameter 1.5–3.5 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 2.2–4.5  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae very numerous, with walls thick and refractive, lumina narrow or apparently lacking, aseptate, occasionally branched, 1.5–3.0  $\mu$  diameter. *Submerged mycelium:* (a) nodose-septate hyphae and (b) fiber hyphae as in aerial mycelium.

**TYPE OF ROT:** white rot of broad-leaved or, rarely, coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Brodie (37), Cartwright and Findlay (52, 56), Davidson, Campbell, and Blaisdell (64), Vandendries (142, 143), Vandendries and Brodie (147, 148).

*Lenzites betulina* has key patterns identical with those of several other species, which makes its recognition in culture difficult. However, in all the cultures examined, nonstaining fiber hyphae were as plentiful in the submerged mycelium as in aerial mycelium, a condition that was not observed in other species. It is hoped that this will prove to be of diagnostic value.

**Lenzites saeplaria** Wulf. ex Fries

KEY PATTERN: (1,2) 2 2 1 9 (1,2) 2 1 (2,3,4) 2 2

## CULTURES EXAMINED:

CANADA.—Quebec: Eagle Depot, on *Pinus Banksiana*, 9528. Ontario: Chalk River, on *Picea glauca*, F3250; Petawawa, on *P. mariana*, 9419. UNITED STATES.—Wisconsin: Rothchild, on *Tsuga* sp., F715. Locality and host not known, F2172. GERMANY.—Eberswalde, on *Pinus sylvestris*, F1007.

CULTURAL CHARACTERS: (Pl. V, Fig. 12; Pl. VI, Figs. 39 to 41).

GROWTH CHARACTERS.—Growth moderately slow to very slow, plates covered in four to seven weeks. Advancing zone somewhat bayed, appressed and hyaline in zone 1.0–2.0 mm. broad. Mat white for two to three weeks, then with patches or zones of "chamois" (2.0Y 7.5/5.8), "honey yellow" (2.0Y 6.7/6.2), "tawny-olive" (8.0YR 4.8/5.8) (three to four weeks), to "snuff brown" (7.0YR 3.9/3.5) (four to six weeks), at first slightly raised, cottony, but soon collapsed, farinaceous, appearing patchy by reason of variations in color and amount of aerial mycelium. Reverse unchanged or somewhat darker below colored areas. Odor slightly spicy. On gallic and tannic acid agars no diffusion zones, trace of growth up to 2.5 cm. diameter on gallic acid agar, no growth or only a trace on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, frequently branched, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, but soon broken up to form oidia; (b) oidia very numerous, cylindric, frequently with swollen portion, or broadly ovoid, often with fragment of clamp connection attached, 5.0–22.0  $\times$  2.5–4.5  $\mu$ . *Submerged mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores rare or apparently lacking, terminal or intercalary, thin-walled, 7.5–16.5  $\times$  5.0–9.0  $\mu$ ; (c) crystals platelike or needlelike.

TYPE OF ROT: brown pocket rot of coniferous or, rarely, broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Cartwright (47), Cartwright and Findlay (51, 54, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Lombard, and Hirt (68), Fries (73), Fritz (74), Humphrey and Siggers (92), Mounce and Macrae (113), Robak (133, 134), Snell (138, 139), Snell, Hutchinson, and Newton (140).

In *Lenzites saeplaria*, the nodose-septate hyphae undergo fragmentation to form oidia. In other species having the same key pattern, oidium formation is restricted to hyphae with simple septa in those portions of the colony that have reverted to a haploid condition. This characteristic makes possible the separation of cultures of *L. saeplaria* from similar species.

**Lenzites trabea** Pers. ex Fries

KEY PATTERN: (1,2) 2 2 1 9 1 2 1 2 1 2

## CULTURES EXAMINED:

CANADA.—Ontario: Ottawa, on lumber from broad-leaved tree, F3823; Toronto, on *Tsuga canadensis*, 9507. UNITED STATES.—Locality and host not known, F3050.

CULTURAL CHARACTERS: (Pl. V, Fig. 13; Pl. VI, Figs. 42 to 46).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, slightly raised aerial mycelium extending to limit of growth. Mat white at first, slightly raised, cottony to woolly, loosely arranged (one week), the older part changing to "cream color" (3.0Y 8.6/4.5), "cream-buff" (3.0Y 8.3/4.5), "warm buff" (1.2Y 7.8/6.0), "chamois" (2.0Y 7.5/5.8), and "cinnamon-buff" (9.0YR 6.6/5.8), cottony-

floccose to woolly, with overgrowth of granular or irregularly pored fruiting surface, "tawny-olive" (8.0YR4.8/5.8) (two weeks), the raised cottony mycelium developing more vivid tones of "pinkish cinnamon" (6.0YR6.5/5.5) and "cinnamon" (5.0YR5.8/6.0) or "buff-yellow" (2.0Y7.9/7.0) to "pale orange-yellow" (1.0Y8.3/6.2), mostly overgrown with irregularly pored or toothed fruiting surface, "Sudan brown" (5.5YR3.8/5.5) and "Brussels brown" (5.0YR3.5/4.0) in color, frequently with brightly colored mycelium grown up side of Petri dish and down between base and lid. Reverse unchanged. Odor very strong, suggesting garlic. On gallic and tannic acid agars no diffusion zones, diameter 2.0 cm. on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, the clamp connections large and conspicuous, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae rare except in fruiting surface, hyaline to buffy brown, with walls thick and refractive except for thin-walled tips, aseptate, rarely branched, 2.2–3.5  $\mu$  diameter; (c) oidia numerous, formed by fragmentation of nodose-septate hyphae, 2.2–3.0  $\mu$  diameter, variable in length. *Fruit body*: (a) nodose-septate hyphae and (b) fiber hyphae as described above; (c) basidia 5.0–6.0  $\mu$  diameter, bearing four spores; (d) basidiospores hyaline, even, cylindric, flattened on one side, 9.0–12.0  $\times$  3.0–4.5  $\mu$ . *Submerged mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores numerous, ovoid to ellipsoid or elongate, intercalary or terminal, with thin walls and contents staining deeply in phloxine, 10.5–18.0 (–28.0)  $\times$  6.0–10.5  $\mu$ .

**TYPE OF ROT**: brown cubical rot of coniferous and broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Badcock (3), Cartwright (49), Cartwright and Findlay (52, 54, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Lombard, and Hirt (68), Humphrey and Siggers (92), Kaufert and Schmitz (94), Mounce and Macrae (113), Snell (138).

The distinctive color, the production of chlamydospores and oidia, and the early fruiting are characteristics that make the key patterns of *Lenzites trabea* unique, and the identification of cultures of the species relatively easy.

### ***Merulius lacrymans* Jacq. ex Fries**

**KEY PATTERNS**: 2 (1,2) 2 1 9 2 2 2 3 2 2 or  
2 (1,2) 2 2 9 2 2 1 4 2 2 ("oidial strain")

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Montcalm, below wooden floor, F3526. Ontario: Ottawa, on beam in cellar, F1606. ENGLAND.—Locality and host not known, F7328, 8787.

**CULTURAL CHARACTERS**: (Pl. V, Figs. 14, 15; Pl. VI, Figs. 48 to 50).

**GROWTH CHARACTERS.**—Growth slow, plates covered in five to six weeks. Advancing zone even or with fans of mycelium extending along edge of Petri dish in advance of mat proper, and then growing in to meet advancing periphery, raised aerial mycelium extending to limit of growth. Mat white at first, then developing patches of "barium yellow" (8.0Y8.0/6.5), "citron yellow" (7.0Y8.0/7.5), and "strontian yellow" (7.0Y8.3/9.0) (three weeks) to "Isabella color" (1.0Y5.5/4.3) (five weeks), slightly raised, silky, then cottony-woolly, with small strands radiating from inoculum over surface of mat and expanding in fanlike arrangement at margin, or with strands grown up sides of Petri dish and over inner surface of cover. Reverse unchanged. Odor strong. On gallic and tannic acid agars no diffusion zones, diameter of colony 1.5–2.0 cm. on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae nodose-septate, 4.5–6.0  $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone, 3.0–7.5  $\mu$  diameter. *Submerged mycelium*: (a) hyphae as in advancing zone, but usually narrower, 2.2–3.5  $\mu$  diameter; (b) crystals numerous, octahedral.

**TYPE OF ROT**: brown cubical rot of coniferous wood.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Badcock (3), Cartwright and Findlay (51, 52, 54, 56), Edgecombe (69), Humphrey and Siggers (92).

The foregoing description of *Merulius lacrymans* is based on only one culture, 8787, which grew as described when first examined in 1939, but which had changed when re-examined in 1942. At the later date it resembled the other cultures of *M. lacrymans* in the collection, all of which had reverted to the haploid condition, characterized by a very slow rate of growth, the radius of the colony being 2.0 cm. or less in six weeks, by the absence of clamp connections on all the hyphae, and by the production of numerous oidia. Cartwright and Findlay (54) quote Falck as having observed a similar change in old cultures of this species, "especially if the culture had been kept for long periods at a temperature above the optimum". The species is included in both the white and colored sections of the key, although the colors are probably always sufficiently deep to warrant its inclusion in the latter group only. Among the colored forms no other species has a key pattern identical with that for *M. lacrymans*. If it is considered as belonging to the white group, with nodose-septate hyphae, then it falls with several other species, from which *M. lacrymans* should be readily separable by reason of its consistent production of vivid yellow patches of mycelium and by its plumose growth. The haploid strain, whether taken as white or colored, stands alone in the key.

### ***Omphalia campanella* Fries**

KEY PATTERN: 2 2 1 1 6 2 2 2 4 2 2

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Lac Vert, on *Abies balsamea*, 10299, on *Picea mariana*, 10298. British Columbia: Big Bend, on *Thuja plicata*, 16124; Clearwater, on *T. plicata*, 16123; Copper Canyon, on *T. plicata*, 16120; Fanny Bay, on *T. plicata*, 16122; Hidden Lake, on *T. plicata*, 11980, 16170, 16622; Port Alberni, on *T. plicata*, 16121; Queen Charlotte Islands, on *Picea sitchensis*, 11981, 11982. UNITED STATES.—On *Thuja plicata*, 11761.

CULTURAL CHARACTERS: (Pl. VII, Fig. 1; Pl. VI, Figs. 51 to 53).

GROWTH CHARACTERS.—Growth very slow, radius 5.0–7.0 cm. in six weeks. Advancing zone even, hyaline and appressed in narrow zone. Mat appressed, at first white, sodden, with sparse aerial mycelium, or cottony-floccose, developing sharply contrasting areas or zones (two to three weeks), crustose, "buckthorn brown" (8.0YR4.8/6.5), "mummy brown" (7.5YR2.5/2.3), and "burnt umber" (2.0YR2.5/2.5), usually with a narrow "ochraceous-tawny" (6.0YR4.9/6.3) border, and with a thin floccose overgrowth of frosty white, from which arise numerous 'aerial rhizomorphs' concolorous with the crustose surface except for whitish tips and "Mars yellow" (6.0YR5.3/10.3) to "Sudan brown" (5.5YR3.8/5.5) tufts of mycelium at their bases, branched, extending into air or meeting surface of agar in advance of mat proper and there developing a velvety brown mycelium that soon produces growth typical of a young colony. Reverse unchanged. Odor none. On gallic and tannic acid agars diffusion zones moderately strong to strong, trace of growth on both media.

HYPHAL CHARACTERS.—Advancing zone: hyphae hyaline, nodose-septate, 1.5–3.0  $\mu$  diameter. Aerial mycelium: (a) nodose-septate hyphae as in advancing zone; (b) in crustose areas hyphae at first hyaline with contents stained in phloxine, then brown and apparently empty, with numerous irregular branches and projections so tightly interlocked that outlines of component parts are lost; (c) conspicuous dark brown hyphae, thick-walled, nodose-septate, 2.2–4.5  $\mu$  diameter, with swellings up to 18.0  $\mu$  diameter. Submerged mycelium: hyphae as in advancing zone.

**TYPE OF ROT:** The formation of brown diffusion zones on media containing gallic or tannic acids by cultures of *Omphalia campanella* suggests that this species produces a white rot, but definite information on this is not available. Buckland (38) states that all of his isolations of *O. campanella* from *Thuja plicata* were from mixed rots, in which it was impossible to determine whether this fungus was the primary cause of decay or only secondary. It is known to occur only on coniferous trees.

If the pseudoparenchymatous layer in *Omphalia campanella* is interpreted as being composed of hyphae with interlocking projections, and listed as "6" under special structures, then the key pattern for this species does not coincide with that for any other species. When it is once known, the purplish-brown crustose areas bristling with the unique 'aerial rhizomorphs' make this one of the most readily recognized species.

### ***Peniophora gigantea* (Fries) Massee**

**KEY PATTERN:** 2 1 (1,2) 2 9 2 2 1 (1,2) 2 2

#### **CULTURES EXAMINED:**

Locality and host not known, F7329, 10249.

**CULTURAL CHARACTERS:** (Pl. VII, Fig. 2; Pl. VIII, Figs. 1 and 2).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, hyaline, appressed. Mat white, all submerged with no aerial mycelium or appressed floccose-farinaceous in more or less extensive areas. Reverse unchanged. Odor strong, penetrating. On gallic acid agar diffusion zones weak to strong, diameter 1.0–2.0 cm.; on tannic acid agar no diffusion zone, no growth.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, the septa usually simple but rarely with large clamp connections, branched, the branch constricted at point of attachment, 2.2–6.0  $\mu$  diameter. *Surface mycelium:* (a) hyphae as in advancing zone; (b) oidia numerous almost to limit of growth, 2.2–4.5  $\mu$  diameter and of varying lengths. *Submerged mycelium:* hyphae as in advancing zone.

**TYPE OF ROT:** pale yellowish-brown rot in sapwood of coniferous logs and worked lumber, according to Cartwright and Findlay (54).

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Biggs (31), Cartwright and Findlay (54, 56), Davidson, Campbell, and Blaisdell (64), Humphrey and Siggers (92).

Clamp connections are so rare in this species that they have been ignored in preparing the key pattern. Three of the four key patterns that must be assigned to the species differ from the patterns for all other species, but one, showing negative reaction on gallic and tannic acid agars and a slower rate of growth, coincides with the key pattern for *Trametes americana*. Among other differences between these two species, the positive reaction of *Peniophora gigantea* on gallic acid agar and negative reaction on tannic acid agar distinguishes it from *Trametes americana*, which shows a negative reaction on both media.

**Pholiota adiposa** Fries

KEY PATTERN: (1,2) (1,2) 1 1 9 2 1 2 (2,3) 2 2

## CULTURES EXAMINED:

CANADA.—Ontario: Ottawa, on *Acer* sp., F969. British Columbia: Aleza Lake, on *Abies lasiocarpa*, 16580; Saanich, on *Abies* sp., 8179, on *Pseudotsuga taxifolia*, 8297; Shawnigan Lake, on *Abies grandis*, 8457. ENGLAND.—Locality and host not known, F1281.

CULTURAL CHARACTERS: (Pl. VII, Fig. 3; Pl. VIII, Figs. 3 to 6).

GROWTH CHARACTERS.—Growth moderately rapid to slow, plates covered in four to five weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white at first, then with tinges of "pale olive-buff" (5.0Y8.2/2.5) to "deep olive-buff" (4.0Y6.7/3.8) (three to four weeks), these greenish-yellow tones changing to yellow—"primrose yellow" (7.0Y8.5/5.2), "colonial buff" (6.0Y8.5/5.5), and "chamois" (2.0Y 7.5/5.8)—in older cultures, at first slightly raised, cottony to woolly, lacunose, becoming collapsed and woolly, with branching and anastomosing strands, deeper in color than surrounding mycelium, conspicuous in vicinity of inoculum. Reverse unchanged in color, with strands in agar prominent. Odor fairly strong, "earthy" (Badcock), like freshly autoclaved soil. On gallic acid agar diffusion zones strong, no growth; on tannic acid agar diffusion zones weak, no growth or only a trace.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, occasionally deep yellow in potassium hydroxide; (b) conidiophores numerous, each being the end of a hypha or short lateral branch, conspicuous by reason of its increased diameter and dense content staining deeply in phloxine, from which the end is cut off by a simple septum to form a conidium, below which the conidiophore may proliferate to produce a branch and second conidium, this continuing until a small fascicle of conidia is formed; (c) conidia plentiful, thin-walled, hyaline to yellow, with dense granular contents, variable in size and shape but typically cylindrical with proximal end straight and distal end rounded, 5.4–11.7  $\times$  2.7–5.4  $\mu$ ; (d) crystals plentiful, needlelike. *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: brown mottled rot of heartwood of living broad-leaved or, more rarely, coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Davidson, Campbell, and Vaughn (67), Edgecombe (69), Mounce (108).

The cultural characters of *Pholiota adiposa* set it apart from all other species included in the present study, but they show it to be similar to *P. aurivella* as described by Martens and Vandendries (104), and as observed in a culture of that species received from the Centraalbureau voor Schimmelcultures at Baarn. Additional studies are necessary to establish a basis for separating these species. One of the key patterns for *P. adiposa* is the same as that for *Polyporus rutilans* but the conidiophores and conidia in the two species are so distinctive as to allow for ready separation.

**Pleurotus ostreatus** Jacq. ex Fries

KEY PATTERN: (1,2) 1 (1,2) 1 9 2 2 2 2 (1,2) 2

## CULTURES EXAMINED:

CANADA.—Ontario: Malakoff, on *Polyporus* sp., 9220; Ottawa, on *Salix* sp., F2503. UNITED STATES.—Locality and host not known, F2949.

CULTURAL CHARACTERS: (Pl. VII, Fig. 4; Pl. VIII, Figs. 7 to 9).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white, slightly raised,

cottony in newest growth, then collapsed, woolly-felty, with slightly raised tufts arranged in more or less concentric zones, producing somewhat zonate appearance, and with small masses of compactly arranged mycelium around edge of Petri dish in some isolates, from which may develop abortive or, occasionally, mature but distorted fruit bodies. Reverse unchanged or bleached after three to four weeks. Odor slight, fragrant. On gallic acid agar diffusion zones moderately strong, on tannic acid agar no diffusion zones, no growth on either medium. (See Davidson, Campbell, and Blaisdell (64).)

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, frequently branched, 2.2–7.5  $\mu$  diameter, usually broad. *Aerial mycelium*: (a) hyphae as in advancing zone, usually 2.2–4.5  $\mu$  diameter, frequently broken up into short segments; (b) fiber hyphae, with walls thick and refractive, the lumina visible only at bases of branches, frequently branched, 1.5–2.2  $\mu$  diameter. *Submerged mycelium*: hyphae as in advancing zone.

**TYPE OF ROT**: white flaky rot of sapwood and heartwood of broad-leaved or, rarely, coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Badcock (3), Cartwright and Findlay (52, 55, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Edgecombe (69), Humphrey and Siggers (92).

The three isolates of *Pleurotus ostreatus* used in this study all gave positive reactions on gallic acid agar, negative reactions on tannic acid agar, but Davidson, Campbell, and Blaisdell (64), using a larger number of cultures, found the most usual reaction on tannic acid agar was a positive one, agreeing with the positive reaction on gallic acid agar. In each of the eight places in which *P. ostreatus* appears in the key it falls with several other species with identical key patterns. In these cases separations can be made only on the basis of differences in macroscopic cultural characters.

### ***Pleurotus ulmarius* Bull. ex Fries**

**KEY PATTERN**: 1 1 2 1 9 2 2 2 (3,4) 2 2

#### **CULTURES EXAMINED**:

CANADA.—Ontario: Ottawa, on *Acer Negundo*, F326, F2418.

**CULTURAL CHARACTERS**: (Pl. VII, Fig. 5; Pl. VIII, Fig. 10).

**GROWTH CHARACTERS.**—Growth slow to very slow, plates covered or radius only 5.0–8.5 cm. in six weeks. Advancing zone even to somewhat bayed, raised aerial mycelium extending to limit of growth. Mat white or very pale "cream color" (3.0Y8.6/4.5) or "cartridge buff" (3.0Y8.5/2.2), raised, loosely arranged, cottony, tangled, sometimes with small drops of colorless exudate over surface. Reverse unchanged. Odor slightly fragrant. On gallic and tannic acid agars no diffusion zones, no growth.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, branched, usually between septa, 1.5–3.0  $\mu$  diameter. *Aerial and submerged mycelium*: hyphae as in advancing zone.

**TYPE OF ROT**: brown rot of living broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Badcock (3), Davidson, Campbell, and Blaisdell (64), Edgecombe (69).

The faster growing cultures of *Pleurotus ulmarius* have a key pattern identical with those for several other species. The loosely arranged, tangled, cottony mat, and lack of growth on media containing gallic and tannic acids make cultures of *P. ulmarius* distinguishable from those of other species in the group.

**Polyporus abietinus** Dicks. ex Fries

KEY PATTERN: (1,2) 1 1 1 1 2 2 2 2 2 2

## CULTURES EXAMINED:

CANADA.—Prince Edward Island: on *Abies balsamea*, F704. Quebec: Alex River, on *A. balsamea*, 11592, 11593, on *Picea mariana*, 11595; Chelsea, on *Pinus Strobus*, F5886; Eagle Depot, on *Larix laricina*, F7072; Lac Vert, on *Abies balsamea*, 10291, on *Picea mariana*, 10289, 10290. Ontario: Ottawa, on *Pinus* sp., F6893. UNITED STATES.—New York: Tupper Lake, on *Picea* sp., F7425. Pennsylvania: Bear Creek, on *Pinus* sp., F1651. Locality and host not known, F380. NORWAY.—Oslo, on *Pinus sylvestris*, F7379.

CULTURAL CHARACTERS: (Pl. VII, Fig. 6; Pl. VIII, Figs. 11 to 14).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, hyaline and appressed in narrow zone. Mat white, in all isolates except F7072 appressed, sparse cottony-floccose to woolly, so thin as to be translucent, frequently with more compact woolly growth in short radiating lines over surface; in F7072 mat raised, cottony, thick enough to be opaque, with surface spongy or honeycombed. Reverse unchanged. No odor. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, frequently branched, 1.5–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone making up most of mat; (b) fiber hyphae with walls thick and refractive, lumina narrow or apparently lacking, 3.0–4.0  $\mu$  diameter, rare or apparently lacking in some isolates, plentiful in F7072; (c) capitate incrustated cystidia numerous, each consisting of a slightly swollen hyphal tip bearing a cap of crystals. *Submerged mycelium*: hyphae as in advancing zone, usually much branched.

TYPE OF ROT: white pocket rot of coniferous or, rarely, broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Cartwright and Findlay (54, 56), Davidson, Campbell, and Blaisdell (64), Fritz (74), Garren (75, 76), Humphrey and Siggers (92), Macrae (103), Robak (133, 134).

Host preferences of *Polyporus abietinus* for coniferous trees and of *P. pargamensis* for broad-leaved trees should be helpful in separating cultures of these similar species, but since both species may occur on both types of hosts other diagnostic characters are needed. The abundance of fiber hyphae in all cultures of *P. pargamensis* and their relative scarcity or apparent absence in most cultures of *P. abietinus* provide the most useful criterion for separation. Caution must be used, however, since one isolate of *P. abietinus* had numerous fiber hyphae and the general appearance of cultures of *P. pargamensis*. This isolate, F7072, was obtained from a lamellate fruit body of *P. abietinus* and monosporous cultures of it were interfertile with other isolates from the lamellate form and, in part, with two isolates of the European poroid form (Macrae (103)). Hence it must be accepted as a culture of *P. abietinus* and its type of growth may be expected in cultures of the species. Thus no definite basis for separating these species in culture is so far available, but in general, cultures with this key pattern from coniferous hosts, showing little aerial mycelium and no or rare fiber hyphae, may be safely assigned to *P. abietinus*.



**Polyporus adustus** Willd. ex Fries

KEY PATTERN: (1,2) 1 2 1 9 2 2 (1,2) 1 2 (2,3)

## CULTURES EXAMINED:

CANADA.—Quebec: Chelsea, on *Fagus grandifolia*, 9208, 9209, on dead stub, 11606; Chute à Blondeau, on *Ulmus americana*, F2271. Ontario: Ottawa, on *Tilia americana*, F1391. UNITED STATES.—Locality and host not known, F2951.

CULTURAL CHARACTERS: (Pl. VII, Fig. 6; Pl. VIII, Figs. 15 to 18)..

GROWTH CHARACTERS.—Growth rapid, plates covered in two weeks. Advancing zone even, slightly raised aerial mycelium extending to limit of growth. Mat white or with tinge of "cream-buff" (3.0Y8.3/4.5) after three to four weeks, cottony-woolly to woolly-floccose in newest growth, finally collapsed and felty, in some isolates so thin as to be translucent. Reverse unchanged or bleached after three to four weeks. Odor slight, "faintly fragrant". On gallic acid agar diffusion zones lacking (may be slight browning of agar), diameter 1.5–3.5 cm.; on tannic acid agar no diffusion zones, no growth.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, profusely branched, 2.2–4.5(–6.0)  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) oidia formed by fragmentation of nodose-septate hyphae or of branches with simple septa, 2.2–3.0  $\mu$  diameter, of lengths varying from 2.0–15.0  $\mu$ . *Submerged mycelium*: hyphae as in advancing zone, frequently irregular, with numerous short branches, projections, and swellings.

TYPE OF ROT: white mottled or cubical rot of broad-leaved or, rarely, coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Bose (32), Cartwright (49), Cartwright and Findlay (51, 56), Davidson, Campbell, and Blaisdell (64), Vandendries (144).

This differs from other descriptions of the species, which have omitted mention of oidia or have described them as being restricted to monosporous cultures (Vandendries (144)), and have reported the occurrence of chlamydo-spores (Cartwright (49)) and of conidia (Bose (32)). In the present study oidia in abundance were observed on nodose-septate hyphae in five of the isolates examined. The remaining isolate (9208) appeared to have lost vigor, in that it produced almost no aerial mycelium and, perhaps correlated with this, no oidia. The other types of secondary spores reported by Cartwright (49) and Bose (32) were not observed, although this apparent discrepancy may be the result of differences in interpretation of the method by which the spores are produced rather than of actual differences in the cultures.

If oidia are observed and their presence included, then the key pattern of *P. adustus* is different from that of any other species. If oidia are omitted, then its key pattern coincides with those for *P. betulinus* and *P. dichrous*, from which separations can be made only on the basis of differences in topography and in the degree of inhibition of growth by gallic and tannic acid agars.

**Polyporus albellus** Peck

KEY PATTERN: 1 1 1 1 (0,9) 1 2 2 2 (1,2) 2

## CULTURES EXAMINED:

CANADA.—Quebec: Chelsea, on *Fagus grandifolia*, F7532; Mt. Burnet, on *Betula lutea*, F6850. Ontario: Caledon East, on *B. lutea*, 10237; Centreton,

on *Betula* sp., 8484; Ottawa, on *B. lutea*, F1394; Petawawa, on *Alnus incana*, 11608.

**CULTURAL CHARACTERS:** (Pl. VII, Fig. 8; Pl. VIII, Figs. 19 to 21).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three weeks. Advancing zone even, hyaline, and appressed or with slightly raised aerial mycelium extending to limit of growth. Mat white, downy at first, after two to three weeks forming overgrowths of cottony-woolly mycelium in loosely arranged balls around inoculum, gradually extending over surface in more or less concentric arrangement, many of the balls becoming waxy and bearing minutely pored fruiting surfaces after four to five weeks. Reverse unchanged. Odor strong and penetrating but not identified. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace to 1.0 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 2.2–4.5  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) contorted hyphal tips, heavily incrustated, conspicuous in mounts, up to 7.0  $\mu$  diameter; (c) chlamydospores fairly numerous, thin-walled, with contents deeply stained in phloxine, terminal or intercalary, 6.0–12.0  $\times$  4.5–7.0  $\mu$ . *Fruit body:* (a) basidia 4.0–5.0  $\mu$  diameter, bearing four spores; (b) basidiospores hyaline, even, allantoid, 3.0–4.0  $\times$  1.5  $\mu$ . *Submerged mycelium:* (a) hyphae as in advancing zone; (b) chlamydospores as in aerial mycelium.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

If the characteristic contorted hyphal tips in cultures of *Polyporus albellus* are recognized and included in the key pattern, then this species falls alone in the key and is readily recognized. If they are omitted, then the key pattern for *P. albellus* coincides with those for several other species, and separation can be made only by considering the whole complex of characters making up each species within the group.

### **Polyporus amorphus** Fries

**KEY PATTERN:** 2 1 1 1 (7,9) 2 2 2 4 (1,2) 2

**CULTURES EXAMINED:**

CANADA.—Quebec: Eagle Depot, on *Pinus Banksiana*, 8550, 8552.

**CULTURAL CHARACTERS:** (Pl. VII, Fig. 9; Pl. VIII, Figs. 22 to 24).

**GROWTH CHARACTERS.**—Growth very slow, radius 3.0–4.0 cm. in six weeks. Advancing zone even, appressed and hyaline in zone 2.0–5.0 mm. wide. Mat white, appressed to slightly raised, compact felty to velvety, with surface somewhat farinaceous, becoming irregularly pored in some isolates. Reverse unchanged. Odor strong, sour. On gallic and tannic acid agars diffusion zones moderately strong to strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, regular in appearance, nodose-septate, 1.5–3.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae numerous, walls thick and refractive, lumina narrow or apparently lacking, unbranched, aseptate, 3.0–4.5  $\mu$  diameter. *Fruit body:* (a) basidia in organized hymenium or in clumps over surface, usually with oily contents that flow from injured cells and obscure mount, 4.0–5.0  $\mu$  diameter, bearing four spores; (b) basidiospores hyaline, even, allantoid, 4.0  $\times$  1.5  $\mu$ . *Submerged mycelium:* hyphae as in advancing zone, frequently with swellings up to 10.0  $\mu$  diameter, at first staining deeply in phloxine, then appearing empty and collapsed.

**TYPE OF ROT:** brown stringy rot of coniferous trees.

Whether or not the swollen cells that occur in the submerged mycelium of *Polyporus amorphus* cultures are included, the key patterns of the species are different from all others in the study. Hence no difficulty should be encountered in recognizing cultures of this species.

**Polyporus anceps** Peck

KEY PATTERN: 2 1 1 1 9 2 2 2 1 1 2

## CULTURES EXAMINED:

CANADA.—Quebec: Aylmer, on *Pinus* sp., F2064. Ontario: Chalk River, on *P. Banksiana*, F3372, F3445; Petawawa, on *Picea* sp., 10213; Port Alexander, on log, 10220. British Columbia: Yellow Point, on *Pseudotsuga taxifolia*, 8264.

CULTURAL CHARACTERS: (Pl. VII, Fig. 10; Pl. VIII, Figs. 25 to 28).

GROWTH CHARACTERS.—Growth rapid, plates covered in two weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white, newest growth raised, cottony-woolly, spongy, becoming appressed and felty in part, with more or less extensive fruiting surfaces formed after two to six weeks, consisting of small compact pored areas or broad zones covered with irregular pores or teeth. Reverse unchanged. Odor none. On gallic and tannic acid agars diffusion zones strong, no growth or only a trace on gallic acid agar, trace to 2.5 cm. on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, frequently branched, 2.2–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae numerous, with walls thick and refractive, lumina narrow or apparently lacking, frequently branched, aseptate, 1.0–2.0(–3.0)  $\mu$  diameter, curving and interwoven to form mat and fruiting surface; (c) dendritically branched hyphae found in F3445 only, mostly in the fruiting area. *Fruit body*: (a) nodose-septate hyphae and (b) fiber hyphae as described above; (c) basidia plentiful even in young cultures, occurring singly, in groups, or in organized hymenium, 6.0–7.5  $\mu$  diameter, bearing four spores; (d) basidiospores hyaline, even, cylindric, 7.5–10.0  $\times$  2.2–3.0  $\mu$ . *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white pocket rot of coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Baxter and Manis (25), Davidson, Campbell, and Blaisdell (64), Mounce (110).

The six isolates of *Polyporus anceps* proved to be so uniform in their cultural characters as to require only one key pattern. This is identical with one of the key patterns for *P. hirsutus*, which, however, is found only rarely on coniferous trees, the exclusive habitat of *P. anceps*. Among the distinguishing characters of the two species, the greater length of the basidiospores of *P. anceps* is the most precise.

**Polyporus arcularius** Batsch ex Fries

KEY PATTERN: 1 2 1 1 6 (1,2) 2 2 1 2 (2,3)

## CULTURES EXAMINED:

CANADA.—Ontario: Ottawa, under *Salix* sp., 10447.

CULTURAL CHARACTERS: (Pl. VII, Fig. 11; Pl. VIII, Figs. 29 to 32).

GROWTH CHARACTERS.—Growth rapid, plates covered in two weeks. Advancing zone even, hyaline and appressed in narrow zone. Mat white (one week), becoming "light brownish drab" (2.0YR4.7/1.8), "avellaneous" (8.0YR6.2/3.5), "vinaceous-fawn" (4.5YR6.7/3.5), with islands remaining white (two weeks), finally "light cinnamon-drab" (2.0YR5.8/1.8), "benzo brown" (2.5YR4.4/1.5), "light brownish drab" (2.0YR4.7/1.8), and "vinaceous-drab" (10.0RP4.7/2.0), mottled, with some areas that remain white, the white parts slightly raised, woolly-felty, the colored areas felty to skinlike, brittle or cartilaginous, wrinkled. Reverse patchy, some areas bleached, remaining areas "honey yellow" (2.0Y6.7/6.2). Odor suggesting honey, and also yeasty. On gallic and tannic acid agars diffusion zones strong, trace of growth on gallic acid agar, 3.5 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–3.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae numerous,

frequently branched, with walls thick and refractive, the lumina visible only at bases of branches, 1.5–3.0  $\mu$  diameter; (c) hyphae from skinlike areas nodose-septate, the walls thickened so that lumina frequently disappear completely, buffy-brown in potassium hydroxide, with numerous short branches or knoblike projections, all interlocked to form a tissue so coherent that it is almost impossible to prepare mounts showing individual elements; (d) chlamydospores rare or apparently lacking, intercalary and terminal, thin-walled, 9.0–13.5  $\times$  6.0–7.5  $\mu$ . *Submerged mycelium*: (a) nodose-septate hyphae and (b) chlamydospores as described above.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64), Refshauge and Proctor (128), Vandendries (145).

This description is based on only one culture, which was isolated from a fruit body having the large pores typical of *Polyporus arcularius*. It resembles closely the cultures of *P. brumalis*, the key patterns of the two species being identical. The one culture of *P. arcularius* available appears to have distinct vinaceous tones and to be darker in color than the cultures of *P. brumalis* but this does not provide a satisfactory basis of separation. A further comparison of these two species in culture is necessary.

### **Polyporus balsameus** Peck

**KEY PATTERN:** 2 1 2 1 9 1 2 2 (2,3) (1,2) (1,2)

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Champlain County, on *Picea mariana*, 10279; Gaspé County, on *Abies balsamea*, F936; Mt. Burnet, on *A. balsamea*, F5624. British Columbia: Aleza Lake, on *A. lasiocarpa*, 16582; Oyster River, on *Tsuga heterophylla*, 8407.

**CULTURAL CHARACTERS:** (Pl. VII, Fig. 12; Pl. VIII, Figs. 33 to 35).

**GROWTH CHARACTERS.**—Growth moderately rapid to slow, plates covered in four to six weeks. Advancing zone even, hyaline and appressed in zone up to 0.5 cm. wide. Mat white at first and remaining so for six weeks or changing to "pinkish buff" (9.0YR7.3/4.5) and "cinnamon" (5.0YR5.8/6.0) in older parts after three to six weeks, cottony, with mycelium erect, loosely interwoven, becoming coarse plumose or tufted in older part and around edge, or with aerial mycelium scanty, appressed, downy, in F936 forming waxy pored surface. Reverse unchanged below white mats, "russet" (2.5YR4.0/3.0) to "cinnamon-brown" (5.0YR3.0/3.0) below colored areas. Odor strong, suggesting iodoform. On gallic and tannic acid agars no diffusion zones (may be slight browning on gallic acid agar), colony 1.5–3.0 cm. diameter on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–6.0 (–7.5)  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores numerous, intercalary or terminal, the latter occurring singly or in spraylike clusters, when immature thin-walled, hyaline, with contents staining in phloxine, then thicker-walled with buffy-brown contents, 7.5–16.5  $\times$  4.5–9.0  $\mu$ . *Submerged mycelium*: (a) nodose-septate hyphae and (b) chlamydospores as in aerial mycelium.

**TYPE OF ROT:** brown butt rot of coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson and Campbell (63), Davidson, Campbell, and Blaisdell (64), Fritz (74), Hubert (90).

The chlamydospores with brown contents in *Polyporus balsameus* are unique, and serve to differentiate this species from all others included in the present study.

**Polyporus Berkeleyi** Fries

**KEY PATTERN:** (1,2) 1 1 2 9 1 (1,2) 2 3 (1,2) 2

**CULTURES EXAMINED:**

**UNITED STATES.**—North Carolina: host not known, F2175. Locality and host not known, F2952.

**CULTURAL CHARACTERS:** (Pl. VII, Fig. 13; Pl. VIII, Figs. 36 to 40).

**GROWTH CHARACTERS.**—Growth slow, plates covered in five weeks. Advancing zone even, hyaline and appressed in narrow zone. Mat white with tinge of "cinnamon-buff" (9.0YR 6.6/5.8) around inoculum (four weeks), the narrow outer zone downy, composed largely of erect conidiophores visible when examined under low magnification, the older part felty and skinlike, with many raised granules over surface, finally uniform, thick, tough, pellicular. Reverse unchanged. Odor strong. On gallic and tannic acid agars diffusion zones strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, with simple septa, branched, the branches usually forming a right angle with the parent hyphae, 2.2–4.5 $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae with walls thick and refractive, lumina narrow but visible and staining in phloxine, branched, with tapering ends, 1.5–3.0 $\mu$  diameter, interwoven to form pellicle; (c) conidiophores most abundant on newest growth and disappearing with formation of pellicle, consisting of erect, swollen hyphae, with contents staining deeply in phloxine, frequently branched, septate, and slightly constricted at the septa, up to 15.0 $\mu$  diameter, each bearing from 1 to 20 or more sterigmata up to 6.0 $\mu$  long, in groups or solitary, at ends or along sides of conidiophores; (d) conidia hyaline, globose, echinulate, apiculate, containing one or more oil globules, 6.0–7.5 $\mu$  diameter. *Submerged mycelium:* (a) hyphae as in advancing zone, up to 7.5 $\mu$  diameter; (b) chlamydospores thin-walled, frequently vacuolate, ovoid, 12.0–16.5  $\times$  7.5–12.0 $\mu$ .

**TYPE OF ROT:** white butt rot of coniferous and broad-leaved trees, especially *Quercus* spp.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67).

The echinulate, globose spores produced by cultures of *Polyporus Berkeleyi* were interpreted as basidiospores by Davidson, Campbell, and Vaughn (67), and by Overholts (Letter, April 17, 1946). This view is supported by the appearance of the spores, which are identical with basidiospores. However, in the preceding description they have been considered as conidia because of their place of production at the margin of the growing colony, because the structures on which they are borne are too irregular to be considered as basidia, and because they are not shot off to form spore deposits when the Petri dishes are inverted. To ensure the recognition of cultures of the species both interpretations have been included in the key. The four key patterns so produced do not coincide with the key patterns for any other species, so that cultures of *P. Berkeleyi* should be readily identifiable.

**Polyporus betulinus** Bull. ex Fries

**KEY PATTERN:** 1 1 2 1 9 2 2 2 (1,2) (1,2) 2

**CULTURES EXAMINED:**

**CANADA.**—Quebec: Farm Point, on stump, F965; Gaspé County, on *Betula* sp., F945; Merrifield's Corners, on *B. lutea*, F1555. Ontario: Ingolf, on *Betula* sp., 9339; Petawawa, on *B. papyrifera*, 9338. British Columbia: Aleza Lake, on *Betula* sp., 16656.

**CULTURAL CHARACTERS:** (Pl. VII, Fig. 14; Pl. VIII, Figs. 41 to 43).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to four weeks. Advancing zone even, composed of well separated, curving, submerged hyphae, limit of growth visible only by transmitted light. Mat colorless to white, slightly raised, cottony to floccose, so thin as to be translucent, becoming collapsed after two to three weeks, with scattered 'dots' of more compact mycelium and, in some isolates, small compact balls usually grown against side wall of Petri dish, bearing fruiting surface. Reverse unchanged. Distinct odor of apples. On gallic and tannic acid agars no diffusion zones, colony 3.0–4.5 cm. diameter on gallic acid agar, 1.7–3.5 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, frequently branched, 1.5–6.0 $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, up to 9.0 $\mu$  diameter, the broader ones frequently having irregularly thickened and refractive walls and lumina empty, the narrower ones with swellings that suggest chlamydospores but which remain thin-walled and eventually become empty and collapsed; (b) fiber hyphae rare except in fruit bodies, with walls thick and refractive, lumina narrow or apparently lacking, rarely branched, aseptate, 1.5–3.0 $\mu$  diameter. *Fruit body:* (a) nodose-septate hyphae and (b) fiber hyphae as described above; (c) basidia 3.5–4.5 $\mu$  diameter, bearing four spores; (d) basidiospores hyaline, even, allantoid, 4.5  $\times$  1.5 $\mu$ . *Submerged mycelium:* (a) hyphae as in advancing zone; (b) crystals numerous, octahedral.

**TYPE OF ROT:** brown cubical rot of sapwood of *Betula* spp.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Cartwright and Findlay (55, 56), Davidson, Campbell, and Blaisdell (64), Macdonald (101).

To separate cultures of *Polyporus betulinus* from other species having the same key pattern, reference must be made to the descriptions of the species involved. None of them have noteworthy characters of diagnostic value but it is hoped that the complex of characters making up each species will be sufficiently distinct to allow for identification. In the case of *P. betulinus* host specificity may be helpful.

***Polyporus borealis* Fries**

**KEY PATTERN:** 2 1 1 1 (0,1) 1 2 2 (2,3) 1 2

**CULTURES EXAMINED:**

**CANADA.**—New Brunswick: St. Andrew's, on *Picea* sp.; F3581. British Columbia: Queen Charlotte Islands, on *Picea sitchensis*, 16070, 16071. Locality and host not known, F582. **ALASKA.**—On *P. sitchensis*, 11685, 11793. **GERMANY.**—Dresden, F1318.

**CULTURAL CHARACTERS:** (Pl. VII, Fig. 15; Pl. VIII, Figs. 44 to 48).

**GROWTH CHARACTERS.**—Growth moderately rapid to slow, plates covered in four to six weeks. Advancing zone even, scant aerial mycelium to limit of growth. Mat white, at first farinaceous, then felty to pellicular (three to four weeks) with more or less conspicuous lines of slightly raised compact mycelium radiating from inoculum, forming foliose or large-pored fruit bodies over raised lumps at edge of Petri dish after four to six weeks. Reverse unchanged. Odor none. On gallic and tannic acid agars diffusion zones weak to strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 2.2–4.5 $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, up to 6.0 $\mu$  diameter; (b) contorted hyphal tips, numerous, irregularly branched, heavily incrustated, 4.5–6.0 $\mu$  diameter; (c) chlamydospores numerous, thick-walled, intercalary and terminal, 12.0–21.0  $\times$  6.0–16.5 $\mu$ . *Fruit body:* (a) basidia 4.5–7.0 $\mu$  diameter, bearing four spores; (b) cystidia fairly numerous, ventricose, 22.0–34.0  $\times$  6.0–7.5 $\mu$ ; (c) basidiospores hyaline, even, ovoid, 4.5–7.0  $\times$  3.5–4.5 $\mu$ . *Submerged mycelium:* (a) hyphae as in advancing zone, up to 7.5 $\mu$  diameter; (b) chlamydospores as in aerial mycelium.

**TYPE OF ROT:** white mottled rot of coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Cartwright and Findlay (56), Davidson, Campbell, and Blaisdell (64), Fritz (74), Robak (132).

Cultures of *Polyporus borealis* are distinctive, with characteristic incrustated contorted hyphal tips and coarsely pored fruiting bodies, which show typical cystidia in the hymenium. Each of the four key patterns, two listing each of these "special structures", stands alone in the key.

**Polyporus brumalis** Pers. ex Fries

**KEY PATTERN:** 1 2 1 1 6 (1,2) 2 2 (1,2) 2 (2,3)

**CULTURES EXAMINED:**

CANADA.—Quebec: Merrifield's Corners, on *Betula lutea*, F1554; Mt. Burnet, on *Ulmus* sp., 9434; Old Chelsea, F1608.

**CULTURAL CHARACTERS:** (Pl. IX, Fig. 1; Pl. VIII, Figs. 49 and 50).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, hyaline and appressed in zone 1.0–2.0 mm. wide. Mat white with scattered areas of "light vinaceous-cinnamon" (6.0YR6.8/4.3), "vinaceous-cinnamon" (6.5YR6.8/5.5), "cinnamon-buff" (9.0YR6.6/5.8), "tawny-olive" (8.0YR4.8/5.8), and "sage brown" (7.0YR5.0/5.5) (two weeks), these increasing in extent and coalescing until whole surface is colored and mottled, or only isolated islands of white remain, the white areas appressed cottony to raised woolly, occasionally sectored, the colored areas skinlike and wrinkled. Reverse unchanged or bleached. Odor faint. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, diameter 2.0–2.5 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 1.5–4.5  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae numerous, with walls thick and refractive, lumina narrow or apparently lacking, aseptate, branched, 1.5–3.0  $\mu$  diameter; (c) hyphae from skinlike areas nodose-septate, with numerous branches, repeatedly branched or with small knoblike projections, so compactly arranged that it is almost impossible to prepare mounts showing individual elements. *Submerged mycelium:* (a) hyphae as in advancing zone, frequently branched; (b) chlamydospores found only in F1554, rare, terminal and intercalary, thin-walled, 9.0–13.5  $\times$  6.0–9.0  $\mu$ .

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

As has been stated under *Polyporus arcularius*, no satisfactory basis for separating that species from *P. brumalis* has been arrived at in the present study, although the vinaceous tones of the former, and the clearer brown color of the latter species may be sufficiently constant to be of diagnostic value. One of the key patterns of *P. brumalis* coincides with those for *Collybia radicata* and *Daedalea confragosa*, but the colors of the mats of these, as noted in the descriptive key, are so different from those shown by *Polyporus brumalis*, as to permit separation.

**Polyporus cinnabarinus** Jacq. ex Fries

**KEY PATTERN:** (1,2) 2 1 1 9 1 2 2 2 1 2

**CULTURES EXAMINED:**

CANADA.—Quebec: Blue Sea Lake, on dead wood, F1377; Old Chelsea, F1609. Ontario: Ottawa, on *Betula lutea*, F1314. UNITED STATES.—Locality and host not known, F2176.

**CULTURAL CHARACTERS:** (Pl. IX, Fig. 2; Pl. X, Figs. 1 to 5).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, hyaline and appressed. Mat white at first, becoming "pinkish cinnamon" (6.0YR6.5/5.5) to "light ochraceous-salmon" (6.0YR 7.2/3.5) (two to six weeks) in more or less extensive areas, with granules and pored areas "zinc orange" (4.0YR5.5/7.0) to "flame scarlet" (2.0YR5.5/10.0) in color (three to six weeks), at first appressed and powdery, then with raised cottony areas, white or colored, over which the brightly colored granules form and coalesce to produce pored fruiting areas (three to four weeks). Reverse unchanged. Odor of green apples in young cultures. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace to 2.0 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 1.5–3.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, frequently distorted, with numerous swellings; (b) fiber hyphae numerous, with walls thick and refractive, lumina narrow or apparently lacking, 2.0–3.0  $\mu$  diameter, curving and interwoven; (c) chlamydospores numerous in older cultures, terminal or intercalary, with walls slightly thickened, 6.0–13.5  $\times$  4.5–9.0  $\mu$ . *Fruit body:* (a) basidia 4.5–6.0  $\mu$  diameter, bearing four spores; (b) basidiospores hyaline, even, short cylindric, 4.5–6.0  $\times$  2.2–3.0  $\mu$ . *Submerged mycelium:* (a) nodose-septate and (b) fiber hyphae, and (c) chlamydospores as described above.

**TYPE OF ROT:** white rot of broad-leaved or, rarely, coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

The vivid "zinc orange" and "flame scarlet" colors in cultures of *Polyporus cinnabarinus*, similar to the colors of the fruit body, make it possible to recognize the species easily, without recourse to microscopic examination and the use of the key.

**Polyporus circinatus** Fries

**KEY PATTERN:** 2 2 1 2 (2,7) (1,2) 2 2 4 2 1

**CULTURES EXAMINED:**

**CANADA.**—Quebec: Champlain County, on *Picea mariana*, 10270, 10271, 10272; Merrifield's Corners, on *Picea* sp., F1557; Notakim Depot, on *P. mariana*, 9416; Chelsea, on *Picea* sp., F987. **ONTARIO:** Timagami, 9825. **UNITED STATES.**—Locality and host not known, F2953.

**CULTURAL CHARACTERS:** (Pl. IX, Fig. 3; Pl. X, Figs. 6 to 10).

**GROWTH CHARACTERS.**—Growth very slow, radius 4.6–7.5 cm. in six weeks. Advancing zone usually even, in some isolates with fans of more rapidly growing mycelium extending beyond margin after four to five weeks, hyaline and appressed in zone 0.2–1.0 cm. broad. Mat "chamois" (2.0Y7.5/5.8), "honey yellow" (2.0Y6.7/6.2), "yellow ocher" (10.0YR 6.8/9.0), "buckthorn brown" (8.0YR4.8/6.5), "Sudan brown" (5.5YR3.8/5.5), and "argus brown" (5.5YR3.3/5.0), appressed, downy to thin velvety, usually not continuous over surface, but aerial mycelium lacking in some areas, the dark brown agar being exposed, frequently with small compact lumps near inoculum or elsewhere, which suggest fruit body fundaments. Reverse "tawny-olive" (8.0YR4.8/5.8), "Dresden brown" (9.0YR3.6/4.0), "Prout's brown" (5.5YR2.8/3.2), "bister" (4.5YR3.0/3.0), the darker colors frequently in irregular zones or patches. Odor none. On gallic and tannic acid agars diffusion zones moderately strong to strong, no growth or only a trace on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, with simple septa, 1.5–4.5  $\mu$  diameter. *Aerial mycelium:* (a) hyphae hyaline to pale yellow or dark brown, with simple septa, branched, 1.5–4.5 (–9.0)  $\mu$  diameter; (b) globose swellings on hyphae frequent, terminal or intercalary, thin-walled, at first hyaline, with contents staining in phloxine, then brown, up to 15.0  $\mu$  diameter; (c) chlamydosporelike bodies fairly numerous in cultures three weeks or more old, hyaline to golden brown, with walls slightly thickened and contents granular or oily in appearance, yellow or brown, usually plasmolyzed to an irregular mass, 7.5–21.0  $\times$  4.5–9.0  $\mu$ ; (d) setae rare or apparently lacking in some isolates, dark brown, sharp pointed, 52.0–60.0  $\times$  7.5–9.0  $\mu$ . *Submerged mycelium:* (a) hyphae as described above, becoming paler with increasing depth in agar; (b) crystals octahedral.



**TYPE OF ROT:** white pocket rot of living coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Cartwright and Findlay (56), Christensen (58), Davidson, Campbell, and Blaisdell (64), Hubert (91).

According to Haddow (80) the proper name for the fungus here treated under *Polyporus circinatus* is *P. tomentosus* Fries. The mechanical difficulty of changing plates of drawings has made it expedient to treat it as originally planned under *P. circinatus*, even while accepting Haddow's argument for the use of the name *P. tomentosus*.

Setae were observed in only one isolate, 10270, which was obtained from a rot and identified as *P. circinatus* on the basis of cultural characters. Since setae were not found in the other cultures examined, some doubt is cast on the accuracy of the identification of this culture, and the question is raised as to whether setae occur in cultures of the species. Structures described as "chlamydosporelike bodies" are numerous and, since they may be interpreted as true chlamydospores, chlamydospores are included in the key patterns for the species. The key patterns without chlamydospores and with swellings or setae fall with *Fomes Pini* together with *Trametes tenuis* or *Poria ferruginosa*. The difficulty of separating cultures of *Fomes Pini* and *Polyporus circinatus* has been noted by Christensen (58) and Hubert (91), who based separation on the "faster growth and more abundant aerial mycelium" of *Fomes Pini* cultures. These macroscopic differences, along with the characteristic chlamydosporelike bodies in *Polyporus circinatus* and the brown thick-walled hyphae with expansions occurring singly or in series in *Fomes Pini*, should provide adequate criteria for the separation of these species.

### ***Polyporus compactus* Overholts**

**KEY PATTERN:** 1 1 1 1 9 1 2 2 (1,2) 1 2

#### **CULTURES EXAMINED:**

CANADA.—Ontario: Constance Bay, on *Quercus rubra*, F7526; Petawawa, on *Q. rubra*, F7525; Port Credit, on *Q. alba*, F637, F7318. UNITED STATES.—Maryland: on *Q. alba*, F8004. Pennsylvania: on *Fagus grandifolia*, F8003.

**CULTURAL CHARACTERS:** (Pl. IX, Fig. 4; Pl. X, Figs. 11 to 15).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, hyaline and appressed in narrow zone. Mat white or with tinges of "massicot yellow" (7.0Y8.1/4.2), coarse cottony and tufted in newer growth, becoming appressed and firm felt to chamoislike in central zone (two weeks), this subsequently overgrown with tufts of mycelium bearing drops of yellow exudate, fruiting after two to three weeks, rarely in pored areas or zones, more commonly heavy spore deposits produced from whole tufted surface. Reverse unchanged. Odor faint in some isolates, strong in others, somewhat fruity. On gallic and tannic acid agars diffusion zones moderately strong to strong, colonies 2.0–4.0 cm. diameter on both media.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, frequently branched, 2.2–4.5(–6.0)  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae with walls thick and refractive, lumina narrow or apparently lacking, aseptate, branched, 1.5–3.0  $\mu$  diameter; (c) chlamydospores numerous, composing most of tufted and pored areas, with fairly thick walls, terminal or intercalary, 8.0–13.5  $\times$  6.0–9.0  $\mu$ . *Fruiting surface:* (a) basidia broad, 12.0–15.0  $\times$  9.0–10.5  $\mu$ , bearing four spores; (b) basidiospores hyaline, even-ovoid, truncate, 6.0–9.0  $\times$  4.5–6.0  $\mu$ . *Submerged mycelium:* (a) hyphae as in advancing zone; (b) chlamydospores as in aerial mycelium, rare.

TYPE OF ROT: white rot of broad-leaved trees, usually *Quercus* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67).

*Polyporus compactus* falls in the numerical key along with several other species, of which only *P. distortus* is so similar as to cause difficulty in separation. Both are characterized by the production of chlamydospores in great abundance, even the apparently well-organized pored areas consisting mainly of these bodies. Basidiospores differ in the two species, and the fact that *P. compactus* is usually on *Quercus* spp. is an additional valuable criterion in separating them.

### **Polyporus conchifer** (Schw.) Fries

KEY PATTERN: 1 1 1 1 9 2 2 2 (1,2) 2 3

#### CULTURES EXAMINED:

CANADA.—Ontario: Ottawa, on *Ulmus* sp., F5033, 9343; Petawawa, on *Ulmus* sp., 10210.

CULTURAL CHARACTERS: (Pl. IX, Fig. 5; Pl. X, Figs. 16 and 17).

GROWTH CHARACTERS.—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, at first hyaline and appressed, later with slightly raised woolly mycelium extending to limit of growth. Mat white, uniform, appressed, subfelty to floccose or somewhat tufted except in parts remote from inoculum where it is raised, woolly, grown against side of Petri dish. Reverse unchanged at first, then bleached. Odor none. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace to 2.0 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae with walls thick and refractive, lumina apparently lacking, 1.5–2.2  $\mu$  diameter, with curving whiplashlike ends, closely interwoven to form tough mat. *Submerged mycelium*: (a) hyphae as in advancing zone, frequently branched; (b) crystals needlelike.

TYPE OF ROT: white rot of dead branches, usually of *Ulmus* spp., rarely of other broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64).

*Polyporus conchifer* has no distinctive cultural characters, and it is difficult to find any criteria by which this species may be differentiated from others with which it coincides in the numerical key. In the descriptive key, the textures of the mats of the different species have been used as a basis for separation. The subfelty to floccose mat of *P. conchifer* and the fact that it usually occurs on dead branches of *Ulmus* spp. may make possible the recognition of cultures of this species.

### **Polyporus cuticularis** Bull. ex Fries

KEY PATTERN: 1 2 1 2 9 2 2 2 (2,3) (1,2) (1,2)

#### CULTURES EXAMINED:

CANADA.—Quebec: Farmer's Rapids, on *Acer* sp., F1969. Ontario: Lake of Bays district, on *Fagus grandifolia*, 10194; Timagami, on *Populus grandidentata*, F756. UNITED STATES.—Locality and host not known, F3552.

**CULTURAL CHARACTERS: (Pl. IX, Fig. 6; Pl. X, Figs. 18 and 19).**

**GROWTH CHARACTERS.**—Growth moderately rapid to very slow, plates covered in four to five (to seven) weeks, the rate of growth varying with the different isolates and with the same isolate at different times, being correlated with the type of growth. In faster growing cultures advancing zone even, with raised aerial mycelium extending to limit of growth; mat white (one to two weeks), to "colonial buff" (6.0Y8.5/5.5), "deep colonial buff" (4.0Y7.7/5.5), and "Isabella color" (1.0Y5.5/4.3) (two to three weeks), to "tawny-olive" (8.0YR4.8/5.8) in some isolates (three to six weeks), the newest growth raised, loosely arranged, cottony, later becoming appressed, woolly to subfelty, culture 10194 after six weeks developing raised lumps of paler mycelium, compactly arranged, surrounded by brown pored areas; reverse unchanged or "honey yellow" (2.0Y6.7/6.2) below newest growth. In slower growing cultures agar "snuff brown" (7.0YR3.9/3.5) to "bister" (4.5YR3.0/3.0) to edge of colony, advancing zone appressed; mat white (one to two weeks), to "straw yellow" (5.0Y9.0/5.5) and "old gold" (3.0Y5.8/5.5) (two to six weeks), cottony, so scanty as to allow dark color of agar to show through. This type of growth may constitute the entire colony or faster-growing, raised, cottony mycelium over pale agar may extend beyond in irregular areas or in a broad zone. No odor. On gallic and tannic acid agars diffusion zones very weak to moderately strong, no growth on gallic acid agar, trace to 2.0 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, with simple septa (rare clamp connections in F1969), frequently branched, 1.5–6.0 $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone, with thin fragile walls, hyaline to pale brown. *Submerged mycelium*: hyphae as in advancing zone.

**TYPE OF ROT**: white stringy rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Cartwright and Findlay (55, 56), Davidson, Campbell, and Blaisdell (64).

In preparing the key patterns for *Polyporus cuticularis* the faster growth rate has been correlated with lack of color in the reverse, and the slower growth rate with color in agar. This has reduced to four the number of times the species appears in the key. In all these places it falls along with several other species, and descriptive keys have been inserted. It should be mentioned that one culture (F1969) showed rare clamp connections in the advancing zone, and two (F1969 and 10194) had setae, although only one or two were found in mounts from each isolate. The occurrence of these structures appears to be too erratic to justify their inclusion in the descriptions or key.

**Polyporus dichrous** Fries

**KEY PATTERN**: (1,2) 1 2 1 9 2 2 2 (1,2) 2 2

**CULTURES EXAMINED**:

CANADA.—Ontario: Petawawa, 8287. British Columbia: Lumby, on *Pinus contorta* var. *latifolia*, 8118. UNITED STATES.—Locality and host not known, F3053.

**CULTURAL CHARACTERS: (Pl. IX, Fig. 7; Pl. X, Fig. 20).**

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, hyaline and appressed, the coarse fibers separated so that limit of growth is difficult to see. Mat hyaline to white, completely submerged and appressed or with some areas developing scanty powdery to floccose or tufted mycelium after two to three weeks. Reverse unchanged or a characteristic greenish-yellow after two to three weeks. Odor strong, disagreeable. On gallic and tannic acid agars no diffusion zones, trace of growth on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–4.0 $\mu$  diameter. *Surface and submerged mycelium*: hyphae as in advancing zone, usually 1.5–3.0 $\mu$  diameter.

**TYPE OF ROT:** white rot of broad-leaved or, rarely, coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

Except for the greenish-yellow color in the agar, and the strong disagreeable odor, cultures of *Polyporus dichrous* are notable only for their complete lack of distinctive characters. In the descriptive keys, *P. dichrous* is separated from other species having the same key patterns by absence of aerial mycelium, lack of fiber hyphae and chlamydospores, and no or only very restricted growth on gallic and tannic acid agars. However, these negative qualities provide satisfactory means of separation.

***Polyporus distortus* (Schw.) Fries**

**KEY PATTERN:** 1 1 1 1 9 1 2 2 1 1 2

**CULTURES EXAMINED:**

**UNITED STATES.**—Mississippi: Locality and host not known, F8002. Locality and host not known, F7992.

**CULTURAL CHARACTERS:** (Pl. IX, Fig. 8; Pl. X, Figs. 21 to 25).

**GROWTH CHARACTERS.**—Growth rapid, plates covered in two weeks. Advancing zone even, hyaline and appressed for 2.0–3.0 mm. Mat white to "light pinkish cinnamon" (7.0YR 7.5/4.5), at first slightly raised, woolly-floccose, then appressed, woolly-felty, with scattered tufts and granules, these coalescing to form compact pored areas, characteristically with cup-like depressions surrounded by pores (three to four weeks). Reverse unchanged. No odor. On gallic and tannic acid agars diffusion zones strong, no growth or only a trace on gallic acid agar, diameter 1.5 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, frequently branched, 1.5–3.0 $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae fairly numerous, with walls thick and refractive, lumina lacking, rarely branched, aseptate, 1.0–2.2 $\mu$  diameter; (c) chlamydospores very numerous even 2–3 mm. from limit of growth, terminal or rarely intercalary, with walls slightly thickened, 7.5–13.5  $\times$  6.0–12.0 $\mu$ ; (d) clavate structures, with contents staining deeply in phloxine, observed only in F7992. *Fruit body:* (a) chlamydospores as described above make up most of mounts from pored areas; (b) basidiospores (from scant spore deposit) hyaline, even, broadly ovoid, apiculate, 5.4  $\times$  3.6 $\mu$ . *Submerged mycelium:* (a) nodose-septate hyphae and (b) chlamydospores as described above; (c) crystals numerous, octahedral, minute.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

The similarity between cultures of *Polyporus distortus* and *P. compactus* has been mentioned in the remarks on the latter species. Differences in basidiospores and the usual occurrence of *P. compactus* on oak serve as distinguishing characters.

***Polyporus dryadeus* Pers. ex Fries**

**KEY PATTERN:** (1,2) 2 1 2 9 2 2 2 4 2 1

**CULTURES EXAMINED:**

Locality and host not known, received from Centraalbureau voor Schimmeltculturen, Baarn, 8101, 9238.

**CULTURAL CHARACTERS:** (Pl. IX, Fig. 9; Pl. X, Figs. 26 and 27).

**GROWTH CHARACTERS.**—Growth very slow, radius 5.0–6.0 cm. in six weeks. Advancing zone even, aerial mycelium uniform to limit of growth. Mat white in border about 0.5 cm. broad, then “yellow ocher” (10.0YR6.8/9.0) to “snuff brown” (7.0YR3.9/3.5) (two weeks), to “old gold” (3.0Y5.8/5.5), “Dresden brown” (9.0YR3.6/4.0), and “Sudan brown” (5.5YR3.8/5.5), in general the white and yellowish-brown areas downy to thin woolly-felty, the reddish-brown areas velvety. Reverse unchanged below newest growth, then “cinnamon-brown” (5.0YR3.0/3.0), “Brussels brown” (5.0YR3.5/4.0) to almost black below oldest part. No odor. On gallic and tannic acid agars diffusion zones moderately strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, frequently branched, with simple septa, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone but some with light brown walls, usually thin-walled and then appearing fragile and frequently distorted in mounts, occasionally with walls slightly thickened, frequently septate, 2.2–4.5  $\mu$  diameter. *Submerged mycelium*: hyphae as in aerial mycelium.

**TYPE OF ROT:** white butt rot of broad-leaved trees, usually *Quercus* spp., and coniferous trees (reported on conifers only from Western United States).

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Cartwright and Findlay (51, 53, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67).

It should be noted that the description of *Polyporus dryadeus* is based on the only two cultures available in the stock culture collection, both of which were received, in 1938 and 1939, from the Bureau voor Schimmelcultures, one accompanied by the information that it had been received from K. St. G. Cartwright of the Forest Products Research Laboratory at Princes Risborough, England. Neither of them has shown the friable or brittle texture or swollen cells described by Davidson, Campbell, and Vaughn (67) and Cartwright and Findlay (53) and so there is some doubt as to whether the cultures are still typical. In the key, *P. dryadeus* falls with several other brown, slow-growing species, but its preference for oaks as host, and the color range of the cultures may be sufficient to identify it.

**Polyporus dryophilus** Berk.

**KEY PATTERN:** 1 (1,2) 1 2 9 2 2 2 (2,3) 2 (1,3)

**CULTURES EXAMINED:**

**UNITED STATES.**—Locality and host not known, F2034, F2035, F2179.

**CULTURAL CHARACTERS:** (Pl. IX, Fig. 10; Pl. X, Figs. 28 to 30).

**GROWTH CHARACTERS.**—Growth moderately rapid to slow, plates covered in four to five weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white (two weeks) to “colonial buff” (6.0Y8.5/5.5) and “chamois” (2.0Y7.5/5.8) (three weeks), and “honey yellow” (2.0Y6.7/6.2) (six weeks); outer zone raised to top of Petri dish, cottony to cottony-woolly, very loosely arranged, the color all in this mycelium; inner zone around inoculum somewhat collapsed, woolly, white or pale. Reverse below newer growth “honey yellow” (2.0Y6.7/6.2) to “bister” (4.5YR3.0/3.0), subsequently bleached, so that in cultures six weeks old agar below oldest part is bleached pure white, below newer growth is “auburn” (3.0YR3.0/4.0) to “bister” (4.5YR3.0/3.0) or nearly black. Odor none. On gallic and tannic acid agars diffusion zones strong, diameter 1.5–2.2 cm. on both media.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, with simple septa, 3.0–7.5  $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone, occasionally with walls yellow to brown. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) crystals numerous, small, octahedral.

**TYPE OF ROT:** white pocket rot of broad-leaved trees, usually *Quercus* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Bailey (4), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Long and Harsch (98).

The colors exhibited by cultures of *Polyporus dryophilus* may be pale, so it seems advisable to include it in both "colored" and "not colored" sections of the key. This possible variation in color, along with two growth rates and the color changes in agar, which necessitates listing under bleached and colored sections, results in *P. dryophilus* appearing in eight places in the key. The color and topography of its cultures are characteristic, and the succession of color changes in the agar, from light to dark brown and finally bleached, is unique, so that it should be possible to separate it readily from other species having identical key patterns.

***Polyporus dryophilus* var. *vulpinus* (Fries) Overholts**

KEY PATTERN: 1 2 1 2 (6,9) 2 2 2 (2,3) (1,2) 2

CULTURES EXAMINED:

CANADA.—Ontario: Constance Bay, on *Populus tremuloides*, F4394; Timagami, on *Populus* sp., F830.

CULTURAL CHARACTERS: (Pl. IX, Fig. 11; Pl. X, Figs. 31 to 35).

GROWTH CHARACTERS.—Growth moderately rapid to slow, plates covered in four to five weeks. Advancing zone even, raised cottony mycelium extending to limit of growth. Mat with broad white margin, changing gradually to "colonial buff" (6.0Y8.5/5.5) and "chamois" (2.0Y7.5/5.8) (three weeks) to "deep colonial buff" (4.0Y7.7/5.5), "honey yellow" (2.0Y 6.7/6.2), and "Isabella color" (1.0Y5.5/4.3) (four weeks), usually paler in older growth around inoculum, raised, cottony to cottony-plumose, collapsed around inoculum and becoming subflety to skinlike, fruiting over inoculum after two weeks (F830), or later in organized, irregularly pored areas or over broad zones where basidia occur in scattered tufts. Reverse unchanged or slightly colored below inoculum, often with "Brussels brown" (5.0YR3.5/4.0) to "Argus brown" (5.5YR3.3/5.0) lines in agar. Odor none. On gallic and tannic acid agars diffusion zones moderately strong, colony 2.0 cm. diameter on gallic acid agar, no growth on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with rare, inconspicuous simple septa, branched, the branches frequently lying parallel to parent hyphae, 2–6.0 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, with septa more numerous, 1.5–4.5 $\mu$  diameter; (b) in skinlike layer at surface of agar hyphae at first hyaline, the contents staining in phloxine, with numerous short curved branches, frequently with knoblike ends, finally with pale yellow walls, and apparently empty, firmly interlocked to form the homogeneous skin, so compact that component parts are no longer apparent. *Fruit body*: (a) basidia in tufts or in continuous hymenium, 6.0 $\mu$  diameter, bearing four spores; (b) basidiospores brown, even, ovoid, 4.5–7.0  $\times$  3.0–4.5 $\mu$ , very abundant. *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white pocket rot of *Populus* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64) (under *Polyporus vulpinus*).

If the pseudoparenchymatous layer is observed and its characteristic cells listed under "special structures" in the numerical key, then *Polyporus dryophilus* var. *vulpinus* keys out alone or with *Hymenochaete tabacina* only, from which it is readily separated on the basis of color and texture. If the cultures do not show these specialized cells, then the key patterns may coincide with those of several other species, of which *Polyporus cuticularis* is particularly close to *P. dryophilus* var. *vulpinus*. The descriptive keys based on

color and topography must be consulted, and consideration must also be given to the fact that *P. dryophilus* var. *vulpinus* is specific to *Populus* spp.

### **Polyporus fibrillosus** Karst.

KEY PATTERN: (1,2) 1 2 2 1 2 2 2 2 1 2

#### **CULTURES EXAMINED:**

CANADA.—Ontario: Petawawa, on *Abies balsamea*, 10909. British Columbia: Nitinat Trail, Vancouver Island, on *Picea sitchensis*, 8201; Queen Charlotte Islands, on *P. sitchensis*, 11708, 16075, on *Tsuga heterophylla*, 16042; Yellow Point, on log, 8238.

CULTURAL CHARACTERS: (Pl. IX, Fig. 12; Pl. X, Figs. 36 to 39).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, hyaline, appressed, limit of growth difficult to discern. Mat white, growth at first submerged, developing more or less extensive areas of appressed to slightly raised floccose to cottony mycelium after two to three weeks, this forming a fruiting surface consisting of granules and incomplete pores mixed with cottony mycelium, from which is produced a heavy spore deposit after five to six weeks. Reverse slightly deeper in color, "primuline yellow" (1.5YR7.5/9.5), "citron yellow" (7.0Y8.0/7.5), and "honey yellow" (2.0Y6.7/6.2), showing through the thin mat. Odor strong. On gallic and tannic acid agars no diffusion zones, diameter 2.0–4.0 cm. on gallic acid agar, no growth on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, frequently branched, with simple septa, 1.5–3.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, up to 4.5  $\mu$  diameter; (b) hyphae with greenish-yellow color, apparently aseptate, branched, 4.5–6.0  $\mu$  diameter. *Fruiting surface*: (a) basidia in compact hymenium or loosely arranged clumps, frequently irregular in shape, about 6.0  $\mu$  diameter, bearing four spores; (b) cystidia numerous, thin-walled, contents staining in phloxine, 3.0–4.5  $\mu$  diameter, projecting up to 45  $\mu$ ; (c) basidiospores hyaline, even, cylindric-ellipsoid, 4.5–6.0  $\times$  3.0  $\mu$ . *Submerged mycelium*: (a) hyphae, hyaline and colored, as in aerial mycelium; (b) crystals large, octahedral.

TYPE OF ROT: brown rot of coniferous or, rarely, broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64).

The combination of characters shown by cultures of *Polyporus fibrillosus* is unique among the species studied, so that its key patterns are unlike any others in the key.

### **Polyporus fragilis** Fries

KEY PATTERN: 2 1 2 1 9 2 2 2 4 2 2

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Gatineau Park, 10227; Mt. Burnet, on conifer, F6874.

CULTURAL CHARACTERS: (Pl. IX, Fig. 13; Pl. X, Figs. 40 and 41).

GROWTH CHARACTERS.—Growth very slow, radius 5.8–6.4 cm. in six weeks. Advancing zone even or slightly bayed, hyaline, appressed. Mat white, appressed, farinaceous to short woolly, so thin as to be translucent except in oldest part. Reverse unchanged. Odor slight at first, strong and bitter in six-weeks-old cultures. On gallic and tannic acid agars no diffusion zones, diameter 1.0 cm. on gallic acid agar, no growth on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone, but frequently broken into segments one or two cells long, and occasionally with swellings. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) crystals octahedral.

TYPE OF ROT: brown rot of coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Cartwright and Findlay (56), Davidson, Campbell, and Blaisdell (64).

The key pattern for *Polyporus fragilis* coincides with that for *Trametes variiformis*, which shows a slow rate of growth and no fruiting. However, the numerous fiber hyphae of the latter species with the resultant felty to pellicular mat, make it decidedly different in appearance from the translucent growth produced by *Polyporus fragilis*.

**Polyporus frondosus** Dicks. ex Fries

KEY PATTERN: 1 1 1 1 9 1 2 2 (2,3) (1,2) (1,2)

CULTURES EXAMINED:

CANADA.—Ontario: Ottawa, 10235. UNITED STATES.—On *Quercus* sp., F2180. Locality and host not known, F2038.

CULTURAL CHARACTERS: (Pl. IX, Fig. 14; Pl. X, Figs. 42 to 46).

GROWTH CHARACTERS.—Growth moderately rapid to slow, plates covered in three to six weeks. Advancing zone even, hyaline and appressed in band up to 0.5 cm. broad. Mat white or with tinge of "chamois" (2.0Y7.5/5.8) and "honey yellow" (2.0Y6.7/6.2) after two weeks, newest growth slightly raised, loosely arranged, woolly, soon becoming compact woolly-felty, thick and opaque, peeling readily from agar, in some isolates forming small pored fruit bodies after five to six weeks. Reverse unchanged or with some areas "tawny-olive" (8.0YR4.8/5.8) to "cinnamon-brown" (5.0YR3.0/3.0). Odor of carbide strong at two to five weeks, disappearing later. On gallic and tannic acid agars diffusion zones weak to strong, diameter trace to 2.5 cm. on gallic acid agar, no growth on tannic acid agar.

HYPHAL CHARACTERS.—Advancing zone: hyphae hyaline, nodose-septate, 1.5–3.0 $\mu$  diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) fiber hyphae fairly numerous or apparently lacking, with walls thick and refractive, lumina very narrow or lacking, branched, 1.5–3.0 $\mu$  diameter; (c) chlamydospores few to abundant, with walls slightly thickened, terminal or intercalary, 10.5–22.0  $\times$  9.0–16.5 $\mu$ . Fruit body: (a) basidia 6.3–7.3 $\mu$  diameter, bearing four spores; (b) basidiospores hyaline, even, apiculate, broadly ovoid, frequently with conspicuous oil drop, 4.5–6.3  $\times$  3.6–4.5 $\mu$ . Submerged mycelium: (a) nodose-septate hyphae and (b) chlamydospores as described above; (c) crystals numerous, large, octahedral.

TYPE OF ROT: spongy butt rot of broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Cartwright (50), Cartwright and Findlay (56), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67).

The coherent felty mat, which peels readily from the agar, and the conspicuously large chlamydospores, together with the characteristic odor of carbide, make cultures of *Polyporus frondosus* readily separable from other species having the same key patterns.

**Polyporus fumosus** Pers. ex Fries

KEY PATTERN: 1 1 (1,2) 1 9 1 2 2 2 (1,2) 2

CULTURES EXAMINED:

CANADA.—British Columbia: Saanichton, on English laurel, 10257. ENGLAND.—Cambridge, 9221.

CULTURAL CHARACTERS: (Pl. IX, Fig. 15; Pl. X, Figs. 47 to 51).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, with cottony fibers scattered over surface to limit of growth. Mat white, slightly raised, cottony, so loosely arranged as to be translucent, with scattered dots



or more or less extensive areas of opaque, subfelty mycelium, which may develop fruiting surfaces, waxy, minutely pored after three to four weeks, producing heavy spore deposits. Reverse unchanged. Odor fairly strong, suggesting turpentine. On gallic and tannic acid agar diffusion zones lacking or very weak, diameter 2.5–3.0 cm. on gallic acid agar, no growth or only a trace on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, frequently broken up into short segments, occasionally with swelling up to 15.0  $\mu$  diameter in older cultures; (b) chlamydospores numerous, intercalary or terminal, with walls somewhat thickened, broadly ovoid to elongate, 7.5–13.5  $\times$  4.5–9.0  $\mu$ . *Fruit body*: (a) basidia 3.6–5.4  $\mu$  diameter, bearing four sterigmata and spores; (b) basidiospores hyaline, even, ellipsoid, or oblong, 3.6–4.5  $\times$  2.7  $\mu$ . *Submerged mycelium*: (a) nodose-septate hyphae and (b) chlamydospores as in aerial mycelium; (c) crystals numerous, octahedral.

**TYPE OF ROT**: white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Badcock (3), Cartwright (49), Cartwright and Findlay (56), Davidson, Campbell, and Blaisdell (64).

Since the key patterns for *Polyporus fumosus* include positive and negative reactions on gallic and tannic acid agars, and presence and absence of fruiting, the species occurs four times in the key, always in association with other species. Its cottony mat, translucent except for dots and larger areas that are subfelty to waxy and may become pored, and its very abundant chlamydospores, should be readily recognizable. Fruit bodies of *P. adustus* and *P. fumosus* are similar, but their cultures are readily separable, those of *P. fumosus* having a moderately rapid growth rate, numerous chlamydospores, and frequent fruiting, and those of *P. adustus* having a rapid growth rate, no chlamydospores but oidia, and no fruiting.

### **Polyporus galactinus Berk.**

**KEY PATTERN**: 1 1 1 3 9 1 2 2 1 2 3

**CULTURES EXAMINED**:

CANADA.—Quebec: Mt. Burnet, on *Populus* sp., F3493, on *Ulmus* sp., F3494.

**CULTURAL CHARACTERS**: (Pl. XI, Fig. 1; Pl. X, Figs. 52 to 54).

**GROWTH CHARACTERS.**—Growth rapid, plates covered in two weeks. Advancing zone even, hyaline, appressed. Mat white, appressed, at first downy, having the appearance of a thin 'bloom' over surface of agar, after two to three weeks patchy, with some areas remaining downy, others becoming thin woolly, with farinaceous surface. Reverse unchanged for three to four weeks, then bleached. Odor faint or lacking. On gallic acid agar diffusion zone strong, no growth; on tannic acid agar diffusion zone weak to moderately strong, diameter up to 2.0 cm.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, at first thin-walled, the walls later becoming conspicuously thickened, branched, the branch constricted at point of emergence from parent hypha, with septa rare, simple, 4.5–6.0  $\mu$  diameter, occasionally giving rise to nodose-septate branches. *Aerial mycelium*: (a) hyphae as in advancing zone rare but conspicuous in mounts, giving off nodose-septate branches; (b) hyphae hyaline, with thin walls, nodose-septate, branched, 1.5–3.0  $\mu$  diameter; (c) chlamydospores very numerous even near margin, intercalary and terminal, with walls slightly thickened, globose to ovoid, 6.0–12.0  $\mu$  diameter. *Submerged mycelium*: (a) nodose-septate hyphae and (b) chlamydospores as in aerial mycelium.

**TYPE OF ROT**: white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Davidson, Campbell, and Blaisdell (64).

The broad, simple-septate hyphae of the advancing zone of *Polyporus galactinus* are striking in appearance and when first observed suggest a faster-growing

contaminant in advance of the nodose-septate hyphae that make up most of the mat. Similar types of hyphae are present in *P. resinosus* and *Poria albipellucida*. Cultures of *Polyporus galactinus* lack the strong fragrant odor of those of *P. resinosus*, and the growth of cultures of *P. galactinus* was inhibited completely on medium containing gallic acid, but good growth on this medium was found in *P. resinosus* and *Poria albipellucida*. These differences aid in the separation of these species.

### **Polyporus gilvus** Schw. ex Fries

KEY PATTERN: (1,2) 2 1 2 (2,9) 2 2 2 2 (1,2) (1,2)

#### **CULTURES EXAMINED:**

UNITED STATES.—New Jersey: Hopewell, on *Acer* sp., F1965. Pennsylvania: State College, on *Quercus* sp., F1704.

CULTURAL CHARACTERS: (Pl. XI, Fig. 2; Pl. XII, Figs. 1 to 6).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white at first, slightly raised, short cottony, with the inoculum and some areas along radiating lines becoming "snuff brown" (7.0YR3.9/3.5), "Saccardo's umber" (9.0YR3.8/3.5), and "sepia" (7.0YR3.4/2.8), more compact, felt to velvety, in F1704 covered with minutely pored fruiting surface after three weeks. Reverse unchanged to "honey yellow" (2.0YR6.7/6.2) and "clay color" (10.0YR5.8/6.0) below colored mycelium. Odor lacking. On gallic and tannic acid agars diffusion zones strong, trace of growth on gallic acid agar, trace to 2.5 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, thin-walled, with simple septa, frequently branched, 2.2–6.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, in part with walls slightly thickened and yellow; (b) hyphae with thick, rigid, brown walls, branched, frequently occurring as isolated cells in otherwise hyaline hyphae, 3.0–6.0  $\mu$  diameter; (c) hyaline, nodose-septate hyphae occasionally observed. *Fruit body*: (a) dark brown hyphae as in aerial mycelium; (b) setae numerous, 20.0–33.0  $\times$  4.5–7.0  $\mu$ ; (c) basidiospores abundant, hyaline, even, oblong-ellipsoid, 4.0–5.0  $\times$  2.0–2.5  $\mu$ . *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white rot of broad-leaved or, rarely, coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Hirt (84), Refshauge and Proctor (128).

In cultures of *Polyporus gilvus* the formation of setae is restricted to the fruiting surfaces. Therefore setae are included under "special structures" only in the key patterns in which fruiting is also listed, and the four key patterns showing this combination of characters fall alone in the key. Hence, when fruiting occurs, there should be little difficulty in identifying cultures of *P. gilvus*. The four key patterns that show no "special structures" and no fruiting are identical with the key patterns for several other species, among which separations must be based on color and texture, as noted in the descriptive keys. In some cultures rare nodose-septate hyphae were observed, but their formation seemed to be too erratic to warrant inclusion in the key.

### **Polyporus glomeratus** Peck

KEY PATTERN: 1 2 1 2 (2,6,9) 2 2 2 4 2 1

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Mt. Burnet, on *Acer rubrum*, F3491, on *Acer* sp., 11254.

### CULTURAL CHARACTERS: (Pl. XI, Fig. 3; Pl. XII, Figs. 7 to 10).

**GROWTH CHARACTERS.**—Growth very slow, radius 3.0–7.5 cm. in six weeks. Advancing zone even, variable, with slightly raised aerial mycelium extending to limit of growth, or appressed and hyaline in broad zone, both types of margin sometimes appearing in different sectors of one colony. Mat frequently patchy, white to "barium yellow" (8.0Y8.0/6.5), "wax yellow" (4.0Y7.8/8.8), "yellow ochre" (10.0YR6.8/9.0), and "olive-yellow" (8.0Y 6.5/6.5), cottony to woolly, sometimes zonate, the aerial mycelium fairly plentiful in some areas, in others so thin that the color is masked by the dark brown color of the agar. Reverse colored to limit of growth in some colonies or sectors, "ochraceous-tawny" (6.0YR4.9/6.3), "cinnamon-brown" (5.0YR3.0/3.0), "mummy brown" (7.5YR2.5/2.3), to almost black. No odor. On gallic and tannic acid agars diffusion zones moderately strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, with simple septa, 2.2–4.5 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) hyphae with walls yellowish-green to pale brown, contents concolorous or dark brown and granular, septate, 2.2–4.5 $\mu$  diameter; (c) in skinlike areas hyphae with numerous short, curved branches, frequently with knoblike ends, with walls pale brown, firmly interlocked to form a pseudoparenchymatous layer; (d) setal hyphae fairly numerous or apparently lacking, dark brown, pointed, 4.5–9.0 $\mu$  diameter, up to 300 $\mu$  long. *Submerged mycelium*: hyphae with yellowish-brown walls and contents, frequently septate, 2.2–4.5 $\mu$  diameter.

**TYPE OF ROT**: white rot and cankers of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Campbell and Davidson (45), Davidson, Campbell, and Blaisdell (64).

If setal hyphae or the special cells comprising the pseudoparenchymatous layer are observed and included in the key patterns, then cultures of *Polyporus glomeratus* key out alone or with two other species having setae distinguishable from the setal hyphae of *P. glomeratus*. However, the species is variable, and cultures may be encountered that show neither of these "special structures". To meet this possibility a key pattern listing no "special structures" has been included for the species. This falls with a large group of species that have been treated in an inserted descriptive key.

### *Polyporus graveolens* Schw. ex Fries

**KEY PATTERN**: 1 (1,2) 1 1 5 2 2 1 2 2 (1,2)

#### CULTURES EXAMINED:

**UNITED STATES.**—New York: Ithaca, F1604. Locality and host not known, F2954.

### CULTURAL CHARACTERS: (Pl. XI, Fig. 4; Pl. XII, Figs. 11 to 14).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, hyaline and appressed. Mat white at first and remaining so (F1604) or changing abruptly after two to three weeks to "saya brown" (7.0YR5.0/5.5) and "cinnamon-brown" (5.0YR3.0/3.0) around inoculum, the colored areas increasing in extent with age, appressed, cottony to subfelty to firm felty. Reverse unchanged for three to four weeks, then with some areas "buckthorn brown" (8.0YR4.8/6.5) to "cinnamon-brown" (5.0YR 3.0/3.0). Odor of pepper (F1604) or musty (F2954). On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace to 1.2 cm. on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–4.5 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, occasionally pale yellow; (b) fiber hyphae with walls slightly thickened but lumina remaining relatively broad, hyaline to greenish-yellow, branched, apparently aseptate, 1.0–3.0 $\mu$  diameter, curving and interwoven; (c) oidia formed by the fragmentation of broader nodose-septate hyphae, frequently with clamp connection attached, 3.0–4.5 $\mu$  diameter and of varying lengths; (d) cuticular cells thin-walled, collapsed and irregular, hyaline to pale brown, compactly arranged and mixed with fiber hyphae to form a pseudoparenchymatous layer. *Submerged mycelium*: hyphae as in advancing zone.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67).

Cultures of *Polyporus graveolens* are unique among those treated in the present study in having a pseudoparenchymatous layer composed of cuticular cells, together with oidia. Davidson, Campbell, and Vaughn (67) describe structures resembling setal hyphae and chlamydospores, neither of which were recognized in the two cultures of the species available to the author for examination, and they omit mention of oidia and cuticular cells. This disagreement may be because of differences in interpretation of the structures observed or it may be due to the fact that the cultures used in the present study, being old, are no longer typical. As described above cultures of *P. graveolens* are similar to those of the species of *Ganoderma* studied in the structure of the pseudoparenchymatous layer and general appearance but differ from them in having oidia.

### **Polyporus guttulatus** Peck

**KEY PATTERN:** (1,2) 2 2 1 9 2 1 2 2 2 2

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Maniwaki, 9418; Old Chelsea, on *Tsuga canadensis*, 11603. Ontario: Timagami, on *Abies balsamea*, F638; locality and host not known, F7331. British Columbia: Oyster River, on *Picea sitchensis*, 9337.

**CULTURAL CHARACTERS:** (Pl. XI, Fig. 5; Pl. XII, Figs. 15 to 17).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in four weeks. Advancing zone even, hyaline, appressed, so that limit of growth is difficult to see. Mat white in newest growth, changing through "vinaceous-buff" (7.0YR7.0/3.5) to "avellaneous" (8.0YR6.2/3.5) and "wood brown" (7.0YR5.7/4.0) (two weeks), to "tawny-olive" (8.0YR 4.8/5.8) and "Saccardo's umber" (9.0YR3.8/3.5) (six weeks), appressed downy to slightly raised cottony-floccose, with drops of amber exudate over surface, older cultures appearing more compact, felty, the surface somewhat reticulate, with farinaceous overgrowth. Reverse unchanged. Odor faint. On gallic and tannic acid agars no diffusion zones, diameter 2.0–3.5 cm. on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 2.2–4.5  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) conidiophores consisting of nodose-septate hyphae, of which the contents of the short terminal cell round up to form a conidium, the clamp connection at its base proliferating to produce a second short terminal cell and conidium, this process continuing until a small terminal head of conidia is produced; (c) conidia numerous, with walls slightly thickened, pale greenish-yellow in potassium hydroxide, contents staining deeply in phloxine, with conspicuous nonstaining granules, broadly ovoid, frequently with basal end flattened, 4.5–7.2  $\times$  2.7–4.5  $\mu$ . *Submerged mycelium:* (a) hyphae as in advancing zone; (b) crystals numerous, octahedral.

**TYPE OF ROT:** brown rot of coniferous or, rarely, broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

The conidia of *Polyporus guttulatus* and their method of formation are so characteristic that there should be no difficulty in recognizing cultures of the species. The only other species in the key in which spores are produced in a comparable manner is *Pholiota adiposa*, but there is no other similarity between the cultures of the two species.

**Polyporus hirsutus** Wulf. ex Fries

KEY PATTERN: (1,2) 1 1 1 9 (1,2) 2 2 (1,2) (1,2) 2

## CULTURES EXAMINED:

CANADA.—Ontario: Ottawa, F1312; Petawawa, on *Crataegus* sp., 10217. British Columbia: Cowichan Lake, on *Alnus* sp., 9884; Oliver district, on *Prunus Armeniaca*, 10240; Vancouver, on *Alnus* sp., F2376. UNITED STATES.—New York: Phoenix, on *Ulmus americana*, F1304. SILESIA.—F1613. SIBERIA.—on *Betula verrucosa*, F1319.

CULTURAL CHARACTERS: (Pl. XI, Fig. 6; Pl. XII, Figs. 18 to 22).

GROWTH CHARACTERS.—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, appressed in narrow zone or with slightly raised aerial mycelium extending to limit of growth. Mat white, at first raised, loosely arranged, cottony-floccose, becoming more compact, woolly to felty or almost plasterlike in older parts, sometimes reticulate, after two or three weeks with balls or large lumps over inoculum or elsewhere, frequently with waxy surface, covered with granules or more or less regular pores, or mycelium growing between Petri dish and cover to produce pored layer there. Reverse unchanged. No odor. On gallic acid agar diffusion zones very strong, no growth; on tannic acid agar diffusion zones moderately strong, diameter of colony 2.0–5.0 cm.

HYPHAL CHARACTERS.—Advancing zone: hyphae hyaline, nodose-septate, 2.2–4.5  $\mu$  diameter. Aerial mycelium: (a) hyphae as in advancing zone; (b) fiber hyphae very numerous, with walls thick and refractive, lumina narrow or apparently lacking, aseptate, branched, 1.5–3.0  $\mu$  diameter. Fruit body: (a) basidia 4.5–6.3  $\mu$  diameter, bearing four spores; (b) basidiospores hyaline, even, cylindric, 5.4–7.2  $\times$  2.2–2.7  $\mu$ . Submerged mycelium: (a) hyphae as in advancing zone; (b) chlamydospores observed in one isolate, terminal and intercalary, thin-walled, 10.5–16.5  $\times$  4.5–7.5  $\mu$ .

TYPE OF ROT: white spongy rot of sapwood of broad-leaved or, rarely, coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Bose (32, 33, 34), Davidson, Campbell, and Blaisdell (64), Humphrey and Siggers (92).

In spite of the possible variations recorded for *Polyporus hirsutus* in the present study it has been found most frequently without chlamydospores and with fruiting, and its common hosts are broad-leaved trees. With this combination of characters it keys out along with *Fomes roseus*, *Pleurotus ostreatus*, and *Schizophyllum commune*, the cultures of which are so distinctive macroscopically as to make possible easy separation. Other key patterns for *Polyporus hirsutus* coincide with those for *P. pubescens* and *P. zonatus*. So far no satisfactory means of separating these similar species has been found. In these and other groups with which *P. hirsutus* falls in the key, separations must be based on a combination of macroscopic and microscopic characters as given in the descriptive keys and in the descriptions.

**Polyporus mollis** Pers. ex Fries

KEY PATTERN: 2 (1,2) 2 2 9 2 2 2 2 2 (1,2)

## CULTURES EXAMINED:

CANADA.—Quebec: Gaspé County, on *Abies balsamea*, F6816, on conifer, F6818, F6827. British Columbia: Cameron Lake, on *Pseudotsuga taxifolia*, 8411. \*

**CULTURAL CHARACTERS:** (Pl. XI, Fig. 7; Pl. XII, Figs. 23 and 24).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in four weeks. Advancing zone even or bayed, appressed, hyaline. Mat white but appearing colored by reason of color in surface layer of agar showing through thin aerial mycelium, "cream color" (3.0YR8.6/4.5), "avellaneous" (8.0YR6.2/3.5), and "wood brown" (7.0YR5.7/4.0) (one week), to "vinaaceous-fawn" (4.5YR6.7/3.5) and "fawn color" (4.0YR5.6/4.2) (two weeks), to "pecan brown" (3.0YR4.7/4.5) (three weeks), the color at first limited to small area around inoculum, later extending over most of plate, the aerial mycelium appressed, downy to sparsely cottony, transparent. Reverse unchanged for two to three weeks, then with color of surface layer showing through. Odor none for two to three weeks, then unpleasant. On gallic and tannic acid agars no diffusion zones, colony 1.5–2.0 cm. diameter on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, with simple septa, 3.0–6.0 $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, frequently branched, containing numerous oil globules or granules; (b) crystals numerous, variable in size, octahedral. *Submerged mycelium:* hyphae and crystals as in aerial mycelium.

**TYPE OF ROT:** brown rot of coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3).

The appearance of color in cultures of *Polyporus mollis* is erratic, and for this reason the fungus is included in both the white and colored sections of the key, and in the sections showing color and not showing color in the agar. Two of the key patterns are unlike those for any other species treated in the present study, while two fall with those for *P. Schweinitzii*, in which the aerial mycelium is distinctly colored in contrast to *P. mollis*, in which the color is confined to the agar.

### **Polyporus montanus** (Quél.) Ferry

**KEY PATTERN:** 2 1 1 2 9 (1,2) 2 2 3 2 2

**CULTURES EXAMINED:**

**CANADA.**—British Columbia: Oyster River, 8268; Queen Charlotte Islands, on *Tsuga heterophylla*, 11149, on ground, 11153; Saanichton, on *Abies grandis*, 8449.

**CULTURAL CHARACTERS:** (Pl. XI, Fig. 8; Pl. XII, Figs. 25 to 27).

**GROWTH CHARACTERS.**—Growth slow, plates covered in five to six weeks. Advancing zone even, appressed and hyaline in narrow zone. Mat white, at first appressed downy, then slightly raised, cottony to subfelty except for some areas or irregular zones in which mycelium is appressed and scanty, giving a somewhat zonate appearance. Reverse unchanged. Odor faint. On gallic and tannic acid agars diffusion zones weak to moderately strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, with simple septa, 1.7–6.0 $\mu$  diameter, usually about 3.0 $\mu$ . *Aerial mycelium:* (a) hyphae as in advancing zone, frequently containing numerous oil globules; (b) fiber hyphae with walls thick and refractive, lumina narrow or apparently lacking, occasionally branched, 1.5–3.0 $\mu$  diameter; (c) chlamydospores rare or apparently lacking, with walls slightly thickened, terminal or intercalary, 9.0–13.5  $\times$  7.5–10.5 $\mu$ . *Submerged mycelium:* (a) hyphae as in advancing zone; (b) crystals large, octahedral.

**TYPE OF ROT:** white laminate rot of coniferous trees.

*Polyporus montanus* was described from Europe and records of its occurrence in North America appear to be rare. Lloyd (97) was of the opinion that it

did not occur on this continent, and that it is the "European analogue of *Polyporus Berkeleyi*" (Lloyd (96)). Dr. L. O. Overholts (Letter, April 17, 1946) stated that it had not been long known in North America but that he had "some fifteen collections from the west coast, including British Columbia . . . . So far as I know it is confined to the west coast". The Department of Agriculture, Mycological Herbarium, Ottawa, contains six collections, all from western conifers, determined as *P. montanus* by Dr. Irene Mounce. Tissue cultures from five of these, and three additional cultures isolated from decays, have been examined. These are somewhat similar to cultures of *P. Berkeleyi*, but lack the echinulate conidia by which cultures of that species are readily recognized. This confirms the opinion that both *P. Berkeleyi* and *P. montanus* occur in North America, and provides additional evidence that *P. montanus* is confined to western conifers.

Cultures of *P. montanus* that have no chlamydospores have a key pattern identical with that for *Poria ferrea*, but since the latter always shows some colored areas its cultures are readily separated from those of the former species.

### ***Polyporus obtusus* Berk.**

KEY PATTERN: 1 1 1 1 9 1 2 2 3 (1,2) 2

#### **CULTURES EXAMINED:**

CANADA.—Ontario: Petawawa, on *Quercus macrocarpa*, F6795; on *Q. rubra*, F6797, F6798, F6800.

CULTURAL CHARACTERS: (Pl. XI, Fig. 9; Pl. XII, Figs. 28 to 31).

GROWTH CHARACTERS.—Growth slow, plates covered in six weeks. Advancing zone even, raised woolly mycelium extending to limit of growth. Mat white, zonate, with zones of raised, woolly, opaque mycelium alternating with zones of appressed, cottony or felty, almost translucent mycelium, giving a 'waved' effect, forming small groups of waxy pores with fimbriate edges on F6798 after six weeks. Reverse unchanged. Odor strong ("Moderately fragrant. Jasmine" according to Badcock (3)). On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, colony 1.0 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–4.5 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, occasionally with numerous branches close together, producing witches' brooms; (b) fiber hyphae with walls thick and refractive, lumina apparently lacking except at tips, aseptate, not branched, 1.5–3.0 $\mu$  diameter; (c) chlamydospores numerous, intercalary and terminal, thin-walled, 6.0–13.5  $\times$  6.0–9.0 $\mu$ . *Submerged mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores as in aerial mycelium; (c) crystals octahedral.

TYPE OF ROT: white rot of broad-leaved trees, usually *Quercus* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67).

Both the key patterns listed above for *Polyporus obtusus* coincide with key patterns for *P. frondosus*, and one also coincides with that for *Poria asiatica*, but the zonate mat of *Polyporus obtusus* cultures, and the numerous chlamydospores that are noticeably smaller than those in *P. frondosus* and *Poria asiatica* should make separations possible.

**Polyporus palustris** Berk. and Curt.

KEY PATTERN: 2 1 2 1 9 1 2 2 2 1 2

## CULTURES EXAMINED:

UNITED STATES.—Locality not known, on *Pinus Taeda*, F2045, on *Pinus* sp., 10618, 10620. Locality and host not known, F2964, F7641, F7642, 10250.

CULTURAL CHARACTERS: (Pl. XI, Fig. 10; Pl. XII, Figs. 32 to 36).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, downy white mycelium extending to limit of growth. Mat white, slightly raised, downy to cottony-floccose, becoming thin felty in the older parts of some isolates, after two to three weeks with scattered, small, raised balls of compact mycelium, these gradually increasing in size and frequently coalescing, with surface at first floccose, later compact and covered with more or less regular pores. Reverse unchanged. Odor faint. On gallic and tannic acid agars no diffusion zones, colonies 2.0–4.5 cm. diameter on both media.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, rare in older cultures; (b) fiber hyphae with walls thick and refractive, lumina narrow or apparently lacking, aseptate, rarely branched, 1.5–3.0  $\mu$  diameter, curving and interwoven; (c) chlamydospores rare, intercalary and terminal, thin-walled, 10.5–19.5  $\times$  7.5–12.0  $\mu$ . *Fruit body*: (a) nodose-septate and (b) fiber hyphae as in aerial mycelium; (c) basidia about 6.0  $\mu$  diameter, bearing four spores; (d) basidiospores hyaline, even, cylindric, flattened on one side, apiculate, 6.0–9.0  $\times$  2.0–3.0  $\mu$ . *Submerged mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores as in aerial mycelium, fairly numerous; (c) broad hyphae with walls slightly thickened, nodose-septate but with clamp connections frequently distorted, contents staining deeply in phloxine, 7.5–9.0 (–15.0)  $\mu$  diameter, seen only in older cultures.

TYPE OF ROT: brown rot of coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64), Nobles (117).

In the key *Polyporus palustris* falls with *Fomes subroseus*, *Polyporus balsameus*, *Poria monticola*, and *P. xantha*. Cultures of *Fomes subroseus* assume characteristic "shell pink" and "vinaceous pink" tones, and the chlamydospores of *Polyporus balsameus* become buffy-brown in color and impart this color to the mat so that cultures of these species are not to be confused with those of *P. palustris*, which remain white. Cultures of *Poria monticola* also remain white or pale but their early formation of foliose fruit bodies, their lack of fiber hyphae, and basidiospores 4.5–6.0  $\times$  2.2–2.7  $\mu$  differ from cultures of *Polyporus palustris*, where fruiting is in compact pored areas, fiber hyphae are numerous, and basidiospores are significantly longer, being 6.0–9.0  $\times$  2.0–3.0  $\mu$ . Like *Poria monticola*, *P. xantha* remains white, and lacks fiber hyphae, and its shorter basidiospores serve to distinguish it from *Polyporus palustris*. In addition, *P. palustris* is southern in its range, and will probably not be encountered in Northern United States or Canada.

**Polyporus pargamensis** Fries

KEY PATTERN: (1,2) 1 1 1 1 2 2 2 2 (1,2) 2

## CULTURES EXAMINED:

CANADA.—Quebec: Chelsea, F5885. Ontario: Constance Bay, on *Populus* sp., F5216; Huntsville, on *Populus* sp., F2373; Timagami, on *P. tremuloides*, F1462. Alberta: Waterways, on *Betula neoalaskana*, F8016.



**CULTURAL CHARACTERS:** (Pl. XI, Fig. 11; Pl. XII, Figs. 37 to 41).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, downy aerial mycelium extending to limit of growth. Mat white, slightly raised, downy to floccose, with areas of raised cottony mycelium, small and scattered or extending over most of surface of some colonies; in F5885 mat thick, felty, papillate, the waxy surfaces of the papillae covered with basidia, which produce a heavy spore deposit in cultures four to five weeks old. Reverse unchanged. Odor none. On gallic and tannic acid agars diffusion zones weak to strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate,  $1.7\text{--}4.5\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae numerous, with walls thick and refractive, lumina apparently lacking, aseptate, not branched,  $2.5\text{--}4.0\mu$  diameter, curving and interwoven; (c) cystidia formed of slightly enlarged hyphal tips each bearing a head of crystals. *Fruit body:* (a) thin-walled and (b) fiber hyphae as in aerial mycelium; (c) cystidia rare, walls somewhat thickened but lumina present and stained in phloxine, usually with cap of crystalline material,  $4.5\mu$  diameter, projecting up to  $25.0\mu$ ; (d) basidiospores hyaline, even, cylindric, slightly curved,  $5.0\text{--}6.5 \times 2.2\mu$ . *Submerged mycelium:* hyphae as in advancing zone.

**TYPE OF ROT:** white rot of broad-leaved or, rarely, coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Macrae (103), Rhoads (130).

The difficulty of separating cultures of *Polyporus abietinus* and *P. par-gamenus* has been discussed under the former species. As was implied there, cultures having the key pattern of these two species, and isolated from decay in broad-leaved trees are more likely to be *P. par-gamenus* than *P. abietinus*.

### **Polyporus pubescens** Schum. ex Fries

**KEY PATTERN:** 1 1 1 1 9 2 2 2 1 (1,2) (2,3)

#### **CULTURES EXAMINED:**

**CANADA.**—Quebec: Eardley, on *Alnus incana*, F7531. Ontario: Chalk River, on *A. incana*, 10214. **UNITED STATES.**—Pennsylvania: Lake Silk-worth, on *A. incana*, F1652. Locality and host not known, F3058.

**CULTURAL CHARACTERS:** (Pl. XI, Fig. 12; Pl. XII, Figs. 42 and 43).

**GROWTH CHARACTERS.**—Growth rapid, plates covered in two weeks. Advancing zone even, with cottony, radiating mycelium extending to limit of growth. Mat white, newest growth cottony, then uniformly felty, more or less pitted, peeling from agar in coherent pellicle, in F7531 growing up side of Petri dish and there forming grayish, translucent, spongy masses without organized pores but producing spore deposits after five weeks. Reverse unchanged or bleached. No odor. On gallic and tannic acid agars diffusion zones moderately strong, no growth on gallic acid agar, colony  $3.0\text{--}4.0$  cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate,  $1.5\text{--}4.5\text{--}(7.5)\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae numerous, with walls thick and refractive, lumina narrow or apparently lacking, branched, the numerous branches usually at right angles to the main hypha, ending in whiplashlike ends,  $1.5\text{--}3.0\mu$  diameter. *Submerged mycelium:* (a) hyphae as in advancing zone; (b) crystals numerous, needlelike.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

In three of its four positions in the key *Polyporus pubescens* coincides with one or more other species having identical key patterns. In the inserted descriptive keys, attempts have been made to separate these on the basis of

distribution of fiber hyphae and topography of the mats, but no differences were observed whereby cultures of *P. pubescens* and *P. hirsutus* could be satisfactorily distinguished.

### **Polyporus radiatus** Sow. ex Fries

KEY PATTERN: 1 2 1 2 (6,9) 2 2 2 2 1

#### CULTURES EXAMINED:

CANADA.—Quebec: Eardley, on *Alnus incana*, F7530; Old Chelsea, on *Acer spicatum*, 10229. Ontario: Ottawa, on *Pyrus* sp., F1565; Petawawa, on *Betula* sp., 10207. UNITED STATES.—New York: Jamesville, on *B. lutea*, F1966. Pennsylvania: Lake Silkworth, on *Betula* sp., F1650. Locality and host not known, received from Bureau voor Schimmelcultures, Baarn, F2286.

CULTURAL CHARACTERS: (Pl. XI, Fig. 13; Pl. XII, Figs. 44 to 46).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, slightly raised aerial mycelium extending to limit of growth. Mat with newest growth white, changing to "colonial buff" (6.0Y8.5/5.5) and "chamois" (2.0Y 7.5/5.8), slightly raised, cottony-woolly, somewhat tufted, the older part "olive-ocher" (3.0Y6.8/6.5), "honey yellow" (2.0Y6.7/6.2), and "Isabella color" (1.0Y5.5/4.3), appressed, short cottony, so sparse as to allow color of surface layers of agar to show through and obscure color of aerial mycelium. Reverse unchanged below newest growth, then "ochraceous-tawny" (6.0YR4.9/6.3), "cinnamon-brown" (5.0YR3.0/3.0), and "Prout's brown" (5.5YR2.8/3.2). Odor faint. On gallic and tannic acid agars diffusion zones weak to moderately strong, growth varying from a trace up to 2.0 cm. diameter on both media.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with simple septa, frequently branched, 1.5–4.5 $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, hyaline to pale brown, frequently septate, 2.2–3.0 $\mu$  diameter; (b) hyphae dark brown, septate, 4.5–7.5 $\mu$  diameter, giving off branches of type (a); (c) surface layers composed of hyphae with numerous short branches, frequently with knoblike ends, in some areas firmly interlocked to produce pseudoparenchymatous layer. *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white rot of broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Cartwright and Findlay (55, 56), Davidson, Campbell, and Blaisdell (64), Humphrey and Siggers (92).

If the characteristic hyphae of the pseudoparenchymatous layer in cultures of *Polyporus radiatus* are observed and included, then the key pattern stands alone in the numerical key, and the culture should be readily determined. If these structures are not present, and the key pattern records no "special structures", then it falls in the key with a large group of colored species. Among these, separations must be made on the basis of color and texture, as set forth in the inserted descriptive key.

### **Polyporus resinosus** Schrad. ex Fries

KEY PATTERN: (1,2) 1 1 3 (8,9) (1,2) 2 2 1 2 (2,3)

#### CULTURES EXAMINED:

CANADA.—Quebec: Chelsea, F1600; Eagle Depot, on *Pinus Banksiana*, 8549. Ontario: Ottawa, F2505, F2506; Peterborough, F2063. UNITED STATES.—New York: Fayetteville, on *Tilia americana*, F1301.

# CULTURAL CHARACTERS: (Pl. XI, Fig. 14; Pl. XII, Figs. 47 to 50).

**GROWTH CHARACTERS.**—Growth rapid, plates covered in two weeks. Advancing zone slightly bayed, appressed, hyaline. Mat white, appressed, farinaceous to thin woolly and subfely, with scattered dots or larger irregular areas in which mycelium is more or less raised, cottony-woolly. Reverse unchanged or bleached after five to six weeks. Odor very strong, sweet, of "equal parts benzaldehyde and anisaldehyde" (Badcock (3)). On gallic and tannic acid agar diffusion zones strong, trace to diameter of 2.0 cm. on gallic acid agar, colony 2.5–3.0 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, frequently branched, the branch constricted at point of emergence from parent hypha, with septa rare, inconspicuous, usually simple but occasionally with clamp connections, 3.0–7.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone limited to newest growth, giving rise to branches 1.5–3.9 (–4.5)  $\mu$  diameter, regularly nodose-septate; (b) "lactiferous cells", conspicuous by reason of their large size and deeply staining contents, irregular in shape and size, up to 30.0  $\mu$  diameter, numerous in some isolates, not observed in others. *Submerged mycelium*: (a) hyphae nodose-septate, frequently branched, 2.2–3.0  $\mu$  diameter; (b) chlamydospores numerous in some isolates, apparently lacking in others, with walls slightly thickened, ovoid, 16.0–24.0  $\times$  9.0–16.5  $\mu$ .

**TYPE OF ROT**: white stringy rot of broad-leaved and coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Badcock (3), Cartwright and Findlay (54), Davidson, Campbell, and Blaisdell (64), Snell, Hutchinson, and Newton (140).

With four characters that may vary, *Polyporus resinusus* has 16 key patterns. The broad, simple-septate hyphae, which give off nodose-septate branches, present in the advancing zone, are distinctive. This type of septation is present also in *Polyporus galactinus*, *Poria albipellucida*, and *P. cinerescens*, all of which have key patterns identical with some of those for *Polyporus resinusus*. The strong fragrant odor of cultures of *P. resinusus*, like that produced by its fruit body, and the conspicuous 'lactiferous cells' present in some isolates of the species, serve to distinguish it from the other species appearing in the key with it.

## *Polyporus rutilans* Pers. ex Fries

**KEY PATTERN**: 1 2 1 1 9 2 1 2 2 (1,2) 2

### CULTURES EXAMINED:

CANADA.—Ontario: Ingolf, 9311, 9312; Petawawa, on *Betula* sp., 11607.

# CULTURAL CHARACTERS: (Pl. XI, Fig. 15; Pl. XII, Figs. 51 to 54).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in four weeks. Advancing zone even, white, aerial mycelium extending to limit of growth. Mat with narrow white border, then "deep colonial buff" (4.0Y7.7/5.5), "amber yellow" (4.0YR4.3/9.5), "chamois" (2.0Y7.5/5.8), and "honey yellow" (2.0Y6.7/6.2) (one week), to "old gold" (3.0Y5.8/5.5), (two weeks), "clay color" (10.0YR5.8/6.0), and "tawny-olive" (8.0YR4.8/5.8) where mycelium has grown up side of Petri dish (five weeks), turning purple when wet with potassium hydroxide solution, at first slightly raised, downy to thin woolly, then farinaceous, as if whole surface were sprinkled with coarse powder, in 11607 forming raised felty areas with pored surfaces after five weeks. Reverse unchanged. Odor strong at first, disappearing with age. On gallic acid agar diffusion zones strong, on tannic acid agar diffusion zones weak, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) conidiophores abundant, especially in young growth, formed from nodose-septate hyphae of which the terminal cells, 30.0–100.0  $\mu$  long, bear closely crowded, short, pointed branches, each with a single, terminal conidium, the whole having a bottle-brush appearance; (c) conidia plentiful, hyaline, globose,

apiculate, 2.0–3.0 $\mu$  diameter; (d) basidia with attached spores occur as ends of main hyphae or short terminal branches on aerial mycelium. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) crystals numerous, large, octahedral.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3).

The conidiophores of *Polyporus rutilans* are so characteristic that there should be no difficulty in recognizing cultures of the species. However, the formation of conidiophores is restricted to a fairly narrow zone of new growth, and they may escape observation if no mounts are taken from this outer region. One other species, *Pholiota adiposa*, has the same key pattern as *Polyporus rutilans* but the methods of forming conidia in the two species are so different that they cannot be confused.

### **Polyporus Schweinitzii Fries**

**KEY PATTERN:** 2 2 2 2 9 (1,2) 2 2 (1,2) 2 (1,2)

**CULTURES EXAMINED:**

CANADA.—Quebec: Chelsea, on *Pinus* sp., F7448; Gaspé County, on *Picea glauca*, F940. Ontario: Ottawa, on *Pinus mughus*, F1897; Timagami, on *P. resinosa*, F855, on *P. Strobus*, F728. British Columbia: Oyster River, on *Picea sitchensis*, 9420, on burned stump, 8270; Saanichton, on *Pseudotsuga taxifolia*, 8284; Vancouver, on *Thuja plicata*, F1039. JAPAN.—Kyoto: F1341.

**CULTURAL CHARACTERS:** (Pl. XIII, Fig. 1; Pl. XIV, Figs. 1 to 5).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to four weeks. Advancing zone even, hyaline and appressed in broad zone. Mat at first hyaline, submerged (one week), then with scattered tufts or small floccose areas "seafoam yellow" (9.0Y9.0/3.0), "chartreuse yellow" (9.0Y8.8/5.0), "primrose yellow" (7.0Y8.5/5.2), "deep colonial buff" (4.0Y7.7/5.5) (two weeks), later variable, with small, raised, soft cottony balls, "amber yellow" (4.0Y7.7/7.0), "primuline yellow" (1.5Y7.5/9.5), "honey yellow" (2.0Y6.7/6.2), or with more extensive velvety zones or irregular patches "raw sienna" (8.0YR5.3/8.5) to "antique brown" (8.0YR4.2/5.5) in color, with large areas of agar lacking aerial mycelium, hyaline to "buckthorn brown" (8.0YR4.8/6.5) and "amber brown" (4.0YR4.3/9.5), frequently with surface of mat appearing metallic or as if covered with shining particles to give a tinsel-like appearance. Reverse unchanged to "primuline yellow" (1.5Y7.5/9.5), "yellow ocher" (10.0YR6.8/9.0), and "buckthorn brown" (8.0YR4.8/6.5). Odor pleasant, "almost anise" (Badcock (3)). On gallic and tannic acid agars no diffusion zones (agar may be reddish-brown below colonies), colony 3.0–5.5 cm. diameter on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, with simple septa, 2.2–6.0 $\mu$  diameter. *Aerial mycelium*: (color in mycelium dissolves in potassium hydroxide solution, turning it greenish-yellow) (a) hyphae as in advancing zone, up to 7.5–9.0 $\mu$  diameter in some isolates; (b) hyphae with walls greenish-yellow and contents concolorous or dark brown in occasional cells, with simple septa, branched, the branches frequently lying parallel to parent hypha, 3.0–6.0 $\mu$  diameter; (c) chlamydospores numerous in some isolates, rare or apparently lacking in others, concolorous with the hyphae in which they are formed, thick-walled, terminal or intercalary, globose to ovoid, 10.5–16.5 $\mu$  diameter. *Submerged mycelium*: (a) hyphae as in advancing zone, up to 9.0 $\mu$  diameter; (b) crystals octahedral.

**TYPE OF ROT:** brown cubical rot of coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Cartwright and Findlay (51, 54, 56), Childs (57), Davidson, Campbell, and Blaisdell (64), Fritz (74), Humphrey and Siggers (92).

The variability of *Polyporus Schweinitzii* in culture has been recorded by Fritz (74), Childs (57), and others, and their observations have been corroborated in the present study. In preparing the description an attempt was made to include the range of characters found in cultures derived from the fruit bodies listed above. However, it should be noted that some isolates from decays grew more slowly, covering the plates only after five to six weeks, and some produced unmistakable diffusion zones on gallic and tannic acid agars, although most of these showed a negative reaction, more usual for the species, in subsequent tests. Since none of the authentic cultures from fruit bodies showed these variations they have been omitted from the description and key.

Boyce (35) and others have stated that *P. Schweinitzii* may occur on broad-leaved trees but such records are so rare that it has been included in the key only in the section on coniferous hosts.

In spite of its variability in culture, *P. Schweinitzii* is readily recognized, it being one of the few colored species showing a negative reaction on gallic and tannic acid agars. Its key patterns coincide only with those of *P. mollis*, in the cultures of which the color is confined to the surface layers of agar, while in cultures of *P. Schweinitzii* the aerial mycelium is definitely colored.

### **Polyporus semipileatus** Peck

KEY PATTERN: (1,2) 1 1 1 0 2 2 2 4 2 (1,2)

#### **CULTURES EXAMINED:**

CANADA.—Ontario: Ottawa, F3499. Locality and host not known, F7335.

CULTURAL CHARACTERS: (Pl. XIII, Fig. 2; Pl. XIV, Figs. 6 and 7).

GROWTH CHARACTERS.—Growth very slow, radius 4.5 cm. or less in six weeks. Advancing zone even, submerged, making it difficult to see limit of growth. Mat white, appressed, farinaceous to thin felty, with scattered dots of more compact mycelium. Reverse unchanged to "cinnamon-brown" (5.0YR3.0/3.0) and "Prout's brown" (5.5YR2.8/3.2). No odor. On gallic and tannic acid agars diffusion zones moderately strong to strong, no growth on either medium.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 2.0–3.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, rare; (b) contorted incrusting hyphal tips, 4.5–6.0  $\mu$  diameter over incrustation, form whole surface. *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white rot of broad-leaved or, rarely, coniferous trees.

The description of *Polyporus semipileatus* was based on the only two cultures available. They are similar and readily separated from other species in the key by reason of the abundant, incrusting, contorted hyphal tips, which make up most of the surface of the cultures, and the slow rate of growth.

### **Polyporus squamosus** Huds. ex Fries

KEY PATTERN: 1 (1,2) 1 1 (6,9) 2 2 1 4 2 2

#### **CULTURES EXAMINED:**

CANADA.—Ontario: Antrim, on *Ulmus americana*, F2274; Ottawa, on *Ulmus* sp., F364, F3512, 10789.

**CULTURAL CHARACTERS:** (Pl. XIII, Fig. 3; Pl. XIV, Figs. 8 to 10).

**GROWTH CHARACTERS.**—Growth very slow, radius 8.5 cm. or less in six weeks. Advancing zone even, mat uniform to limit of growth. Mat white, with or without small areas "snuff brown" (7.0YR3.9/3.5) to "drab" (9.0YR5.5/2.0) around inoculum, appressed, fibers fine, short, straight, recumbent, producing a uniform surface except for occasional skinlike areas or zones in which superficial aerial mycelium is completely lacking. Reverse unchanged. Odor none. On gallic and tannic acid agars diffusion zones weak to moderately strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae nodose-septate, 2.2–4.5 $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) oidia very numerous, formed 2–3 mm. inside limit of growth, by the fragmentation of nodose-septate hyphae, with dense granular contents, variable in shape from narrow-elongate to broadly-ellipsoid, 3.0–6.0 $\mu$  diameter, up to 45.0 $\mu$  in length; (c) in skinlike layer at surface of older parts of colony, hyphae with walls slightly thickened and refractive, septate, with numerous nodules and irregular protuberances, compactly arranged to form a pseudoparenchymatous layer. *Submerged mycelium:* hyphae as in advancing zone.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Cartwright and Findlay (55, 56), Davidson, Campbell, and Blaisdell (64), Vandendries (146).

If the characteristic cells of the pseudoparenchymatous layer are observed and listed in the key pattern, or if the color of the culture is sufficiently pronounced to warrant its inclusion in the section treating cultures with colored mats, then the key patterns for *Polyporus squamosus* fall alone in the key. A culture that remains white and shows no pseudoparenchymatous layer keys out with *P. umbellatus* and there appears to be no means of distinguishing between cultures of these two species.

### ***Polyporus subcartilagineus* Overholts**

**KEY PATTERN:** (1,2) 1 2 1 9 2 2 2 3 2 2

**CULTURES EXAMINED:**

**CANADA.**—Quebec: Notakim Depot, north of Maniwaki, on *Picea mariana*, 9417.

**CULTURAL CHARACTERS:** (Pl. XIII, Fig. 4; Pl. XIV, Fig. 11).

**GROWTH CHARACTERS.**—Growth slow, plates covered in five weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white, raised, cobwebby-cottony to plumose, becoming collapsed and appressed in central part, but remaining raised around edge of Petri dish. Reverse unchanged. No odor. On gallic and tannic acid agars no diffusion zones, colony 2.0 cm. diameter on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone, aerial mycelium,* and *submerged mycelium:* hyphae all uniform, hyaline, nodose-septate, 2.2–6.0 $\mu$  diameter.

**TYPE OF ROT:** brown carbonizing rot of broad-leaved and coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson and Campbell (63).

The only culture of *Polyporus subcartilagineus* in the collection was isolated from the rot accompanying a fruit body on *Picea mariana*, collected in the same location as the type but in the following year. Since the species is a recently described one (Overholts (121)) it seems advisable to include a description of its cultural characters even though it is based on only one culture. Davidson and Campbell (63) described cultures of the species isolated from black cherry and their cultures appear similar to the one used in the present study although no chlamydospores were observed in the latter

isolate. In both appearances in the key *P. subcartilagineus* falls with several other species from which it may be separated by reason of its raised cottony to plumose initial growth and its lack of fiber hyphae.

***Polyporus sulphureus* Bull. ex Fries**

KEY PATTERN: (1,2) 1 2 2 9 1 (1,2) 2 2 2 2

**CULTURES EXAMINED:**

CANADA.—Ontario: Ottawa, on *Quercus* sp., F1543. British Columbia: Queen Charlotte Islands, on *Picea sitchensis*, 11710, 11711. UNITED STATES.—Pennsylvania: on *Quercus* sp., F2183. Locality not known, on *Quercus* sp., F2046.

CULTURAL CHARACTERS: (Pl. XIII, Fig. 5; Pl. XIV, Figs. 12 to 15).

GROWTH CHARACTERS.—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, appressed, with scattered tufts of mycelium over zone of newest growth, 1.0–2.0 cm. broad. Mat white to "pale ochraceous-buff" (8.5YR8.0/3.5) and "pale ochraceous-salmon" (9.0YR8.2/2.5), slightly raised, floccose-farinaceous. Reverse unchanged. Odor none. On gallic and tannic acid agars no diffusion zones, colonies 3.0–4.0 cm. diameter on both media.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with simple septa, 3.0–9.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, usually 3.0–6.0  $\mu$  diameter, the few broader hyphae conspicuous; (b) conidiophores numerous, making up most of aerial mycelium, 2.2–3.0  $\mu$  diameter, branched in spraylike manner, bearing a single conidium at end of each branch; (c) conidia numerous, thin-walled, broadly ovoid to subglobose, 6.0–9.0  $\times$  6.0–7.5  $\mu$ ; (d) chlamydospores fairly numerous, with walls slightly thickened, terminal and intercalary, 12.0–19.5  $\times$  7.5–13.5  $\mu$ . *Submerged mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores as in aerial mycelium.

TYPE OF ROT: brown cubical rot of broad-leaved and coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Badcock (3), Cartwright and Findlay (51, 53, 54, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Fritz (74), Humphrey and Siggers (92).

Fritz (74) and Davidson, Campbell, and Vaughn (67) considered all the secondary spores of *Polyporus sulphureus* to be chlamydospores, although they noted that those borne on the aerial mycelium are smaller than those produced on the submerged hyphae. On the other hand, Cartwright and Findlay (53) called the spores on the aerial mycelium conidia, and this interpretation has been followed in the above description, while the larger spores of the submerged mycelium have been considered as typical chlamydospores. However, to meet the possibility of the secondary spores all being taken as chlamydospores, key patterns for the species are included, which show only chlamydospores, and both chlamydospores and conidia. Each of the four resulting key patterns occurs alone in the key so there should be no difficulty in determining cultures of *P. sulphureus*.

***Polyporus tuberaster* Jacq. ex Fries**

KEY PATTERN: (1,2) 2 1 1 (6,9) 2 2 2 (2,3) 2 1

**CULTURES EXAMINED:**

CANADA.—Manitoba: Raeburn, F2349. Alberta: Grande Prairie, F984. ITALY.—Avellino, F7997. Locality and host not known, received from Centraalbureau voor Schimmelcultures, Baarn, F6623.

**CULTURAL CHARACTERS:** (Pl. XIII, Fig. 6; Pl. XIV, Figs. 16 to 18).

**GROWTH CHARACTERS.**—Growth moderately rapid to slow, plates covered in four to six weeks. Advancing zone even, aerial mycelium to limit of growth. Mat white at first, becoming "pinkish buff" (9.0YR 7.3/4.5), "cinnamon-buff" (9.0YR 6.6/5.8), "tawny-olive" (8.0YR 4.8/5.8), "snuff brown" (7.0YR 3.9/3.5), "avellaneous" (8.0YR 6.2/3.5), and "buffy-brown" (9.0YR 4.6/3.5), the color usually restricted to an area around the inoculum or to certain zones, appressed, downy to subfelty, then felty to pellicular or, in colored area around inoculum, with glazed surface almost lacking aerial mycelium. Reverse unchanged for one to two weeks, then with scattered lines or patches of "snuff brown" (7.0YR 3.9/3.5) to "bister" (4.5YR 3.0/3.0) below colored areas in mat. No odor. On gallic and tannic acid agars diffusion zones moderately strong to strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 1.5–4.5  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae numerous, frequently branched, thick-walled, lumina visible only at bases of branches, 1.5–3.0  $\mu$  diameter; (c) in skinlike areas hyphae nodose-septate, with numerous short branches or irregular protuberances, having thick refractive walls, all closely interwoven with fiber hyphae to form a pseudoparenchymatous layer. *Submerged mycelium:* hyphae as in advancing zone.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64), Mounce (110).

Fruit bodies of *Polyporus tuberaster* grow from an underground sclerotium-like structure, which may enclose foreign material such as stones and portions of roots (Güssow (77)). There is no suggestion that the species can cause decay. It is included in the present study merely because of its academic interest and to record that isolates received from Europe appear to be identical with those isolated from sclerotia or the fruit bodies produced therefrom collected in Western Canada. Since there is no provision in the key for species of fungi that are not wood-inhabiting, *P. tuberaster* has been included with the species occurring on both broad-leaved and coniferous trees. Having two other variable characters it occurs eight times, and in two places coincides with the key patterns for *Daedalea confragosa*. The rarity and restricted known range for *Polyporus tuberaster* makes it extremely unlikely that this species will be encountered among cultures to be identified.

**Polyporus Tulipiferae** (Schw.) Overholts

**KEY PATTERN:** 1 1 (1,2) 2 9 2 2 2 (1,2) 2 3

**CULTURES EXAMINED:**

**CANADA.**—Ontario: Ottawa, on *Sorbus Aria*, F8048, on *Quercus* sp., F8049, on *Rosa* sp., F8047. British Columbia: Summerland, on *Prunus Armeniaca*, 10233. **UNITED STATES.**—Locality and host not known, F369, F2958.

**CULTURAL CHARACTERS:** (Pl. XIII, Fig. 7; Pl. XIV, Figs. 19 and 20).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, slightly raised aerial mycelium extending to limit of growth. Mat white, slightly raised, cottony to floccose-cottony, with aerial mycelium more abundant in some areas, raised, spongy-woolly, somewhat tufted. Reverse bleached. Odor strong, disagreeable but not identified, becoming less noticeable in cultures five to six weeks old. On gallic and tannic acid agars diffusion zones lacking or weak, colony 2.5–6.5 cm. diameter on gallic acid agar, no growth or colony up to 1.5 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, with simple septa, 3.0–6.0  $\mu$  diameter. *Aerial mycelium:* hyphae as in advancing zone, 1.5–6.0  $\mu$  diameter, most frequently 1.5–3.0  $\mu$ ; (b) fiber hyphae numerous in some isolates, rare or apparently lacking in others, with walls thick and refractive but lumina visible, rarely septate, occasionally branched, 2.0–3.0  $\mu$  diameter. *Submerged mycelium:* hyphae as in advancing zone.



**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Darley and Christensen (61), Davidson, Campbell, and Blaisdell (64).

Three of the key patterns for *Polyporus Tulipiferae* occur alone in the key, while the fourth coincides with that for *P. dryophilus*. Cultures of *P. Tulipiferae* remain white, while those of *P. dryophilus* assume yellow or buff colors that may be pale but are distinct. Hence there should be no difficulty in separating cultures of the two species.

***Polyporus umbellatus* Pers. ex Fries**

**KEY PATTERN:** 1 1 1 1 9 2 2 1 4 2 (1,2)

**CULTURES EXAMINED:**

CANADA.—Ontario: Petawawa, 9263. Locality and host not known, received from Centraalbureau voor Schimmelcultures, Baarn, F7336.

**CULTURAL CHARACTERS:** (Pl. XIII, Fig. 8; Pl. XIV, Figs. 21 and 22).

**GROWTH CHARACTERS.**—Growth very slow, radius 4.7 cm. or less in six weeks. Advancing zone slightly bayed, aerial mycelium uniform to limit of growth. Mat white, appressed, compact, velvety to coarse farinaceous, appearance suggesting rough plaster. Reverse unchanged or "honey yellow" (2.0Y6.7/6.2) to "cinnamon-brown" (5.0YR3.0/3.0) below inoculum. Odor faint. On gallic and tannic acid agars diffusion zones very weak to moderately strong, no growth on either medium.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, the cells short, with numerous branches originating at and between septa, 3.0–4.5  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, soon fragmented to form (b) oidia of diameter equal to hyphae and of varying lengths, frequently with clamp connection attached, 9.0–15.0  $\times$  3.0–4.5  $\mu$ . *Submerged mycelium:* hyphae as in advancing zone.

**TYPE OF ROT:** white rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64).

*Polyporus umbellatus* and *P. squamosus* fall together in the key and there appears to be no clearly defined character by which they may be separated.

***Polyporus versicolor* L. ex Fries**

**KEY PATTERN:** (1,2) 1 1 1 9 (1,2) 2 2 (1,2) 2 3

**CULTURES EXAMINED:**

CANADA.—British Columbia: Saanichton, on *Arbutus Menziesii*, 8182, on *Juglans* sp., 8183; Summerland, on *Prunus* sp., 10019; Vancouver, on *Cornus Nuttallii*, 10021.

**CULTURAL CHARACTERS:** (Pl. XIII, Fig. 9; Pl. XIV, Figs. 23 to 25).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, hyaline, appressed for short distance in advance of aerial mycelium. Mat white with tinge of "cream color" (3.0Y8.6/4.5), raised cottony-woolly in newer growth and remaining so in some areas, otherwise felty, with drops of exudate that may leave the surface punctate on their disappearance, mycelium grown up sides of Petri dish and across cover after three weeks. Reverse bleached after three to four weeks. Odor strong, "fish and tallow" according to Badcock (3). On gallic and tannic acid agars diffusion zones moderately strong, trace of growth to colony 1.5 cm. diameter on gallic acid agar, colony 2.5–3.0 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 3.0–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae numerous, thick-walled, lumina discernible only at bases of branches, frequently branched, 2.0–3.0  $\mu$  diameter, curving and interwoven; (c) chlamydospores fairly numerous in some isolates, usually found lying free in preparations for microscopic examination, thin-walled, 4.5–7.5  $\times$  2.2–4.5  $\mu$ . *Submerged mycelium*: hyphae as in advancing zone.

**TYPE OF ROT**: white spongy rot of broad-leaved and, rarely, coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Badcock (3), Bayliss (27), Cartwright and Findlay (51, 52, 55, 56), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Fritz (74), Humphrey and Siggers (92), Jay (93), Refshauge and Proctor (128), Vandendries and Brodie (147, 148).

Six of the eight key patterns assigned to *Polyporus versicolor* fall with others in the key, in groups that are difficult to separate. The chlamydospores of *P. versicolor* are significantly smaller than those of other species in the groups, and this character, along with the distinctive, strong 'fishy' odor and the cottony-woolly to felty mat, which often becomes punctate, may permit recognition of cultures of this species.

### **Polyporus volvatus** Peck

**KEY PATTERN**: 2 1 1 1 7 2 2 2 (2,3) 2 2

**CULTURES EXAMINED**:

CANADA.—Quebec: Gaspé County, on *Picea glauca*, F6814, 10188. UNITED STATES.—Locality and host not known, F3060.

**CULTURAL CHARACTERS**: (Pl. XIII, Fig. 10; Pl. XIV, Figs. 26 to 28).

**GROWTH CHARACTERS.**—Growth moderately rapid to slow, plates covered in three to six weeks. Advancing zone even, appressed so that it is difficult to see limit of growth. In F6814 and 10188 growth mostly submerged, sodden, translucent except for some opaque dots within or at the surface of the agar, where they may coalesce, especially in the vicinity of the inoculum, to form opaque areas with irregular 'bubbly' surface, in F3060 mat white, appressed, thin farinaceous. Reverse unchanged. Odor pungent. On gallic and tannic acid agars diffusion zones moderately strong, no growth on gallic acid agar, trace of growth to diameter of 1.0 cm. on tannic acid agar (Davidson, Campbell, and Blaisdell (64)).

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate, 1.5–4.5  $\mu$  diameter. *Aerial and submerged mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae (observed only in F3060) with walls thick and refractive, frequently branched, 1.5–2.2  $\mu$  diameter; (c) hyphae with swellings, usually in moniliform arrangement, nodose-septate, the clamps being small and inconspicuous, with granular contents, very numerous, especially in opaque areas, 7.5–15.0  $\mu$  diameter.

**TYPE OF ROT**: white rot of coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Davidson, Campbell, and Blaisdell (64), Zeller (153).

The sodden appearance of the cultures and the minute granules floating in the mounting medium suggest the presence of a bacterial contaminant but since all the cultures, including some isolated from decays and not listed above, had this appearance, it has been accepted as typical for the species. The characteristic hyphae with swellings are of diagnostic value, being found only in this one species of all those included in the key. The "conidiospores" described by Zeller (153) were not observed in any of the cultures available.

**Polyporus zonatus** Fries

KEY PATTERN: 1 1 1 1 9 1 2 2 1 2 (2,3)

## CULTURES EXAMINED:

NORWAY.—Oslo, on *Alnus incana*, F7388, on *Populus tremula*, F7389, on *Salix caprea*, F7390. Locality and host not known, received from Bureau voor Schimmelcultures, Baarn, F2280.

CULTURAL CHARACTERS: (Pl. XIII, Figs. 11, 12; Pl. XIV, Figs. 29 to 31).

GROWTH CHARACTERS.—Growth rapid, plates covered in two weeks. Advancing zone even, in some isolates appressed and hyaline so that it is difficult to see limit of growth, in others with the raised aerial mycelium extending to limit of growth. Mat white, the first growth slightly raised, woolly-floccose, becoming collapsed, farinaceous to thin woolly, later the whole surface appressed, felty to pellicular, sometimes appearing rough or pebbled. Reverse unchanged or bleached after two to three weeks. Odor none or slight. On gallic and tannic acid agars diffusion zones moderately strong to strong, no growth on gallic acid agar, colony 1.5–3.3 cm. diameter on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–4.5(–6.0)  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in the advancing zone, the septa usually close together and the branches numerous, in some isolates with narrow staghorn branches; (b) fiber hyphae numerous, with walls thick and refractive and lumina apparently lacking, frequently branched, 1.5–3.0  $\mu$  diameter; (c) chlamydospores fairly numerous to rare, terminal and intercalary, thin-walled, 6.0–12.0  $\times$  4.5–7.5(–9.0)  $\mu$ . *Submerged mycelium*: (a) nodose-septate hyphae and (b) chlamydospores as described above; (c) crystals numerous, slender, needle-like.

TYPE OF ROT: white rot of broad-leaved trees.

The description of *Polyporus zonatus* is based on four cultures received from Europe, there being no isolates from American specimens in the stock culture collection. Its inclusion at least puts on record the cultural characters of *P. zonatus* as it is known in Europe, and may permit comparison of these cultures with American isolates of the species. *P. zonatus* coincides in the key with *P. versicolor*, and cultures of the two species appear so similar as to be indistinguishable.

**Poria albidellucida** Baxter

KEY PATTERN: (1,2) 1 1 3 9 1 2 (1,2) 1 2 (2,3)

## CULTURES EXAMINED:

CANADA.—British Columbia: Cowichan Lake, on *Thuja plicata*, 9264, 9285, 11692; Newton, on *T. plicata*, 8246; Saanichton, on *Pseudotsuga taxifolia*, 9757; locality not known, on *Thuja plicata*, 11605; neither locality nor host known, 11268.

CULTURAL CHARACTERS: (Pl. XV, Fig. 1; Pl. XVI, Figs. 1 to 5).

GROWTH CHARACTERS.—Growth rapid, plates covered in one to two weeks. Advancing zone even, hyaline, appressed. Mat white, at first appressed or submerged, with aerial mycelium thin downy or almost lacking, translucent except for numerous scattered V-shaped areas, with points directed away from inoculum, opaque because of denser growth within agar and fine woolly to farinaceous surface, later with overgrowth of slightly raised woolly or cottony mycelium beginning around edge of Petri dish and growing inward, in some isolates covering most of surface, in others restricted to band around outside. Reverse unchanged or partially bleached, the V-shaped thickenings prominent. No odor. On gallic acid agar diffusion zone very strong, colony 5.0–8.0 cm. diameter; on tannic acid agar diffusion zone moderately strong, colony trace to 1.5 cm. diameter.

**HYPHAL CHARACTERS.**—*Advancing zone*: leading hyphae hyaline, thin-walled at first but walls soon becoming thickened, with rare, inconspicuous, simple septa,  $3.0\text{--}7.5\mu$  diameter, frequently branched, the branches soon developing clamp connections. *Aerial mycelium*: (a) thick-walled hyphae with simple septa as in advancing zone, rare but conspicuous; (b) nodose-septate hyphae,  $1.5\text{--}3.0\mu$  diameter; (c) chlamydospores usually numerous, terminal and intercalary, globose and subglobose,  $7.5\text{--}10.5\mu$  diameter; (d) oidia rare in some isolates, apparently lacking in others, formed in advancing zone by fragmentation of hyphae with simple septa,  $4.5\text{--}6.0\mu$  diameter, of varying lengths. *Submerged mycelium*: (a) nodose-septate hyphae and (b) rare chlamydospores as described above.

**TYPE OF ROT**: white laminate rot of coniferous and broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Buckland (38).

Morphological differences in the fruit bodies of *Poria albipellucida* and *P. cinerescens*, particularly in the basidiospores, which are ovate,  $4.5\text{--}5.0 \times 3.5\text{--}4.5\mu$  in the former (Cooke (60)) and cylindric,  $4.5\text{--}6.0 \times 1.5\text{--}2.5\mu$  in the latter (Overholts (122)) establish the existence of two species, but separation of the cultures on the basis of the diagnostic characters employed in the present study, has proved impossible. For this reason the two species have been listed together in the key, and no description or illustrations of *P. cinerescens* have been included, since they appear to be identical with those given for *P. albipellucida*. In the key *P. albipellucida* coincides with *Polyporus resinosus* in three places, with *P. galactinus* in one. The characteristic V-shaped thicker areas in the mat of *Poria albipellucida* provide a recognizable feature, and the lack of odor in this species separates it from the fragrant cultures of *Polyporus resinosus*. The rapid growth of cultures of *Poria albipellucida* on medium containing gallic acid serves to separate them from cultures of *Polyporus galactinus* in which growth is completely inhibited by gallic acid agar.

***Poria asiatica*** (Pilát) Overholts

**KEY PATTERN**: (1,2) 1 (1,2) 1 9 1 2 2 (3,4) 2 2

**CULTURES EXAMINED**:

CANADA.—British Columbia: Clearwater, on *Thuja plicata*, 17119; Hidden Lake, on *T. plicata*, 17121; Sooke, on *T. plicata*, 11700; Upper Thompson district, on *T. plicata*, 17118, 17120; locality not known, on *T. plicata*, 11753, 11754.

**CULTURAL CHARACTERS**: (Pl. XV, Fig. 2; Pl. XVI, Figs. 6 and 7).

**GROWTH CHARACTERS.**—Growth slow to very slow, plates covered in five to six weeks or radius  $5.4\text{--}8.4$  cm. in six weeks. Advancing zone even, raised aerial mycelium uniform to limit of growth. Mat white, raised, cottony-floccose in newer growth, becoming more compact, woolly-felty, this type of growth extending uniformly over colony or interrupted by areas with scanty aerial mycelium, to produce a patchy appearance, with roll of cottony mycelium grown against glass in vicinity of inoculum in some isolates. Reverse unchanged. Odor of iodoform strong in most isolates when young, disappearing after three weeks. On gallic acid agar no diffusion zones, colony  $1.2\text{--}2.3$  cm. diameter; on tannic acid agar diffusion zones lacking or very weak, trace of growth to colony  $1.3$  cm. in diameter.

**HYPHAL CHARACTERS.**—*Advancing zone*: hyphae hyaline, nodose-septate,  $2.2\text{--}4.5\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores very numerous in most isolates, terminal and intercalary, the walls at first thin, becoming thickened and frequently rough, variable in size and shape,  $7.5\text{--}21.0 \times 4.5\text{--}12.0\mu$ . *Submerged mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores as in aerial mycelium; (c) crystalline material plentiful.

**TYPE OF ROT:** brown cubical butt and trunk rot of coniferous or, rarely, broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Buckland (38).

The chlamydospores of *Poria asiatica*, with thick, rough walls, are unique, and their occurrence makes possible the ready recognition of cultures of this species. In most other respects cultures of *P. asiatica* are remarkably similar to those of *Polyporus balsameus*, but the chlamydospores of the latter species become buffy brown in culture, and usually are sufficiently plentiful to impart this color to the mat. The key patterns of *Poria asiatica* also coincide with those for several other species, but the peculiarities of the chlamydospores permit ready separation from these species.

### ***Poria carbonica* Overholts**

**KEY PATTERN:** 2 1 2 1 4 1 1 2 (2,3) 2 2

#### **CULTURES EXAMINED:**

CANADA.—British Columbia: Cameron Lake, on *Pseudotsuga taxifolia*, 8248, 8250; Cowichan Lake, 8215; Ladysmith, 8254, 8277; Mud Bay, 8255; Oyster River, 8269, 8271, 8278; Royston, 8247; Saanichton, 8281, 8282; Westholme, 8256, all on *P. taxifolia*.

**CULTURAL CHARACTERS:** (Pl. XV, Fig. 3; Pl. XVI, Figs. 6 to 9).

**GROWTH CHARACTERS.**—Growth moderately rapid to slow, plates covered in four to six weeks. Advancing zone even, hyaline and appressed in zone up to 1.0 cm. wide. Mat white or with tinges of "pale chalcedony yellow" (9.0Y4.5/8.3); appressed, downy to woolly-felty, after three to four weeks producing raised balls of mycelium with cottony or velvety surfaces, along radii or scattered. Reverse unchanged. Odor of apples. On gallic and tannic acid agars no diffusion zones, colony 2.0–3.0 cm. diameter on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 1.5–3.0(–6.0)  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) conspicuous much-branched hyphae, the branches usually attached at right angles and frequently rebranched, the walls slightly thickened and rigid, lumina fairly broad and apparently empty, aseptate, 3.0–6.0  $\mu$  diameter; (c) conidia numerous, borne singly at the tips of branches, which are usually narrower than the main hyphae, about 1.5  $\mu$  diameter, thin-walled, broadly ovoid, slightly truncate at distal end, pointed at attached end, 7.0–9.0  $\times$  4.5–7.0  $\mu$ ; (d) chlamydospores numerous, intercalary and terminal, walls slightly thickened, broadly ovoid, 9.0–16.5  $\times$  7.5–12.0  $\mu$ . *Submerged mycelium:* (a) hyphae as in advancing zone; (b) chlamydospores as in aerial mycelium; (c) crystals numerous, octahedral.

**TYPE OF ROT:** brown rot of western coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Nobles (117).

The key patterns for *Poria carbonica*, showing chlamydospores, conidia, and the much-branched rigid hyphae known only in this species, stand alone in the key and the species is readily identified. Although they do not conflict in the key, mention should be made of the similarity between cultures of *Fomes officinalis* and *Poria carbonica*, both of which produce large numbers of chlamydospores and conidia. However, the special hyphae of *P. carbonica* and its more rapid rate of growth are characters by which the two species are separated in the key. In addition, *P. carbonica* has been reported only from British Columbia, Washington, and Oregon, so that it appears to be restricted to Western Canada and United States.

***Poria cinerescens* (Bres.) Sacc.**

KEY PATTERN: (1,2) 1 1 3 9 1 2 (1,2) 2 (2,3)

## CULTURES EXAMINED:

CANADA.—British Columbia: Cowichan Lake, on *Thuja plicata*, 9267, 9877; Saanichton, on *Prunus emarginata*, 17072.

The three authentic cultures of *Poria cinerescens* available for study appeared to be identical with cultures of *P. albipellucida*, and therefore, as noted under that species, a description of *P. cinerescens* has been omitted. Until means of separation have been established, cultures showing the characteristics of these species cannot be identified with certainty.

***Poria colorea* Overholts and Englerth**

See *Poria subacida*.

***Poria ferrea* (Pers.) Bourd. and Galz.**

KEY PATTERN: (1,2) (1,2) 1 2 9 2 2 2 3 2 (2,3)

## CULTURES EXAMINED:

CANADA.—British Columbia: Duncan, on *Pseudotsuga taxifolia*, 9250; Saanichton, on *Alnus* sp., 8461, 9254, on *Pseudotsuga taxifolia*, 8243, 9256.

CULTURAL CHARACTERS: (Pl. XV, Fig. 4; Pl. XVI, Figs. 12 and 13).

GROWTH CHARACTERS.—Growth slow, plates covered in five weeks. Advancing zone even, appressed or with slightly raised tufts of aerial mycelium. Mat white with tinges of "tawny-olive" (8.0YR4.8/5.8) to "buckthorn brown" (8.0YR4.8/6.5) over inoculum, and after three to four weeks in scattered flecks or narrow zones, appressed, cottony, hyphae recumbent and radiating, sometimes grouped in slightly raised fans. Reverse unchanged for three to four weeks, then bleached. No odor. On gallic and tannic acid agars diffusion zones strong, no growth to trace on gallic acid agar, colony 1.0–2.5 cm. on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with simple septa, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) hyphae with walls thin to slightly thickened, pale yellow to "Sudan brown" (5.5YR3.8/5.5), some frequently septate, others with long whiplashlike ends, aseptate, branched, 1.5–4.5  $\mu$  diameter. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) crystals numerous, octahedral.

TYPE OF ROT: white rot of broad-leaved and coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Baxter (12, 15).

*Poria ferrea* is usually described as occurring on broad-leaved trees but a number of collections in the Mycological Herbarium, Department of Agriculture, Ottawa, are from the coniferous species *Pseudotsuga taxifolia* and *Thuja plicata*. Baxter (15, p. 278) also encountered this species on conifers, and has listed cultures from *Abies* sp. and *Thuja plicata*. It is noteworthy that these reports of the occurrence of *Poria ferrea* on coniferous hosts are all from the west coast areas, British Columbia and Oregon.

Since there are three variable key characters in cultures of *Poria ferrea*, host, color of mat, and color of reverse, the species appears in eight places in the key. In four of these positions it falls with one or more other species but none of these is so similar to *P. ferrea* as to make separation difficult. In each case a descriptive key has been inserted in which differential characters are set forth.

***Poria ferrugineo-fusca* Karst.**

KEY PATTERN: 2 2 1 2 2 2 2 3 2 2

## CULTURES EXAMINED:

CANADA.—Ontario: Timagami, on *Picea glauca*, F1411. British Columbia: Aleza Lake, on *Abies lasiocarpa*, 16614, on *Picea glauca*, 16621. Locality and host not known, received from Bureau voor Schimmelcultures, Baarn, F7340.

CULTURAL CHARACTERS: (Pl. XV, Fig. 5; Pl. XVI, Figs. 14 and 15).

GROWTH CHARACTERS.—Growth slow, plates covered in five weeks. Advancing zone even, appressed, hyaline. Mat "avellaneous" (8.0YR6.2/3.5) and "wood brown" (7.0YR5.7/4.0) (one week), with overgrowth of "honey yellow" (2.0Y6.7/5.2) to "ochraceous-tawny" (6.0YR4.9/6.3) (six weeks), at first with aerial mycelium scanty, then cottony, finally compact woolly with scattered small balls or granules, producing rough surface, with drops of colorless exudate. Reverse unchanged. Odor none. On gallic acid agar diffusion zone strong, on tannic acid agar diffusion zone very weak to moderately strong, no growth or only a trace on either medium.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with occasional simple septa, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) setal hyphae very numerous, giving color to mat, pale greenish-yellow to "cinnamon-brown" (5.0YR 3.0/3.0) and "Prout's brown" (5.5YR2.8/3.2) in potassium hydroxide, long, flexible, curving, 4.5–6.0  $\mu$  diameter, tapering to sharp point. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) crystals numerous, octahedral.

TYPE OF ROT: yellow ring rot of coniferous trees.

*Poria ferrugineo-fusca* falls close to *P. Weirii* in the key, but is distinguishable from it by its slower growth rate. The topography of the cultures of the two species differs also, the mat of *P. ferrugineo-fusca* being thin in newer growth, raised in the older part, while that of *P. Weirii* is raised in the newer part, and tends to become collapsed in older parts. These differences in type of growth result in noteworthy differences in appearance of mature cultures.

***Poria ferruginosa* (Schröd. ex Fries) Karst.**

KEY PATTERN: (1,2) 2 1 2 2 2 2 2 4 2 1

## CULTURES EXAMINED:

CANADA.—Quebec: Mt. Burnet, on broad-leaved tree, 11253. UNITED STATES.—Massachusetts: October Mountain, on *Fraxinus* sp., 9513.

CULTURAL CHARACTERS: (Pl. XV, Fig. 6; Pl. XVI, Figs. 16 to 18).

GROWTH CHARACTERS.—Growth very slow, radius 4.0–7.0 cm. in six weeks. Advancing zone with shallow bays, the aerial mycelium uniform to limit of growth. Mat white at first and remaining so over most of surface or only in narrow zone, changing to "honey yellow" (2.0Y6.7/6.2), "sandal brown" (7.0YR5.0/5.5), "Saccardo's umber" (9.0YR3.8/3.5), with flecks of "ochraceous-tawny" (6.0YR4.9/6.3) (one to six weeks) over most of surface or in restricted zone around inoculum, appressed, downy to thin felty, occasionally with slightly more compact growth in fan-shaped areas. Reverse unchanged below white parts of mat, "ochraceous-tawny" (6.0YR4.9/6.3) to "buckthorn brown" (8.0YR4.8/6.5) below colored areas. No odor. On gallic and tannic acid agars diffusion zones moderately strong to strong, trace of growth to colony 1.0 cm. diameter on both media.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with simple septa, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, hyaline to dilute brown, frequently branched and anastomosed; (b) setae or setal hyphae numerous, thick-walled, dark brown, pointed, elongate, 90.0–200.0  $\times$  4.5–6.0  $\mu$ , frequently the end of a dark brown hypha. *Submerged mycelium*: hyphae as in advancing zone.

TYPE OF ROT: white rot of broad-leaved and, rarely, coniferous trees.

The elongate setae or setal hyphae of *Poria ferruginosa* are distinct from the short setae of *Fomes Pini* and *Polyporus circinatus*, and also from the broader setal hyphae of *Polyporus glomeratus*, cultures of which, in addition, exhibit a pseudoparenchymatous layer not found in *Poria ferruginosa*. It is possible, therefore, to separate *P. ferruginosa* from these other species having identical key patterns.

### *Poria microspora* Overholts

See *Poria monticola*.

### *Poria monticola* Murr.

KEY PATTERN: (1,2) 1 2 1 9 1 2 2 (1,2) (1,2) 2

#### CULTURES EXAMINED:

CANADA.—British Columbia: Queen Charlotte Islands, on *Picea sitchensis*, F347, F458, 10724, 10726, 11712, 11713, 11714; Vancouver, on *Tsuga heterophylla*, 9251. Original locality not known, isolated from decay in building timber (*Pinus Banksiana*), 8191 (*Pinus* sp.), 11620, 11179 (*Pseudotsuga taxifolia*), F7640, 9176, 9429, 9511 (coniferous timber), F1280, F8009.

CULTURAL CHARACTERS: (Pl. XV, Fig. 7; Pl. XVI, Figs. 19 to 24).

GROWTH CHARACTERS.—Growth rapid to moderately rapid, plates covered in two to four weeks. Advancing zone even, appressed and hyaline in some isolates, or with raised aerial mycelium extending to limit of growth. Mat white or with tinge of pale "cartridge buff" (3.0YR 5/2.2) to "cinnamon-buff" (9.0YR 6.6/5.8) at edges, slightly raised, loosely arranged, cottony to woolly, frequently grown against sides of Petri dish and across lid, forming scattered fruit bodies after two to three weeks in some isolates, these consisting of waxy granules or plates coalescing to form foliose or, more rarely, irregularly pored fruiting surfaces, which produce copious spore deposits. Reverse unchanged. Odor none. On gallic and tannic acid agars no diffusion zones, diameter 3.0–5.6 cm. on gallic acid agar, no growth or only a trace on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–6.0  $\mu$  diameter. *Aerial mycelium*: (a) nodose-septate hyphae as in advancing zone, in some isolates occasionally up to 8.0–16.5  $\mu$  diameter, with walls irregularly thickened and lumina relatively narrow; (b) chlamydospores rare to numerous, with walls slightly thickened, contents staining deeply in phloxine, intercalary or terminal, 8.0–19.5  $\times$  6.0–13.5  $\mu$ . *Fruit body*: (a) nodose-septate hyphae as in advancing zone; (b) basidia 13.0–18.0  $\times$  4.5–6.3  $\mu$ , bearing four sterigmata and spores; (c) basidiospores hyaline, even, oblong-ellipsoid, apiculate, somewhat flattened on one side, 4.5–6.3  $\times$  2.2–2.7  $\mu$ . *Submerged mycelium*: (a) hyphae as in advancing zone; (b) hyphae with thick walls and irregular swellings up to 16.5  $\mu$  diameter, found only in older cultures; (c) crystals numerous, octahedral.

TYPE OF ROT: brown cubical rot of coniferous and broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Cartwright (48, under *Trametes serialis*), Cartwright and Findlay (54, under *T. serialis*; 56, under *Poria microspora*), Nobles (117, under *Poria microspora*), Snell (139, under *Trametes serialis*).

This fungus, described as *Poria microspora* by Overholts in a paper by the author (Nobles (117)), was subsequently found by Overholts (124) to be identical with a part of the type collection of *Poria monticola* Murr. Hence the name *Poria microspora* must be reduced to synonymy under *Poria monticola*.



The faintly pinkish tinge of the mycelium, frequently too pale to be identified in Ridgway's scale, and the early formation of characteristic foliose fruit bodies with heavy spore deposits in most isolates, serve to differentiate this fungus from other species. A few isolates produced an abundant growth of aerial mycelium and failed to form fruit bodies during the period of examination. In the key these have been separated from other species with identical key patterns on the basis of color and type of chlamydospores.

*Poria monticola* may persist in wood in service and prove to be very destructive (Hirt and Lowe (86)). It is frequently encountered among cultures isolated from decay in building timbers.

***Poria obliqua*** (Pers. ex Fries) Bres.

KEY PATTERN: 1 2 1 2 2 2 2 3 1 (1,2)

CULTURES EXAMINED:

CANADA.—Nova Scotia: Kentville, on *Betula* sp., F2277. New Brunswick: Fredericton, on *B. lutea*, F1384. Quebec: Mt. St. Gregoire, on *B. lutea*, F5558. Ontario: Kathmore, on *Ostrya virginiana*, F7403; Petawawa, on *Betula papyrifera*, F7449. UNITED STATES.—Pennsylvania: Hunt County, on *Betula* sp., F1705.

CULTURAL CHARACTERS: (Pl. XV, Fig. 8; Pl. XVI, Figs. 25 to 29).

GROWTH CHARACTERS.—Growth slow, plates covered in five to six weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white (one week), the older part becoming "straw yellow" (5.0Y9.0/5.5) and "reed yellow" (7.0Y7.8/6.2) (two weeks), to "amber yellow" (4.0Y7.7/7.0), "mustard yellow" (2.0Y7.8/7.5), and "antimony yellow" (1.0Y6.5/7.0) (three weeks), the newest growth raised, loosely arranged, cottony, the older part in some isolates collapsed and so thin as to allow color of agar to show through, in other isolates thin feltly and opaque, fruiting after four to six weeks in more compact raised areas, scattered or in zones, "yellow ochre" (10.0YR6.8/9.0) to "buckthorn brown" (8.0YR 4.8/6.5), with surface irregularly pored. Reverse in some isolates unchanged, in others "buckthorn brown" (8.0YR4.8/6.5) to "argus brown" (5.5YR3.3/5.0) below oldest parts. On gallic and tannic acid agars diffusion zones very weak to weak, no growth on gallic acid agar, none to trace on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with thin walls and simple septa, 2.0–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, "gradually grading into yellowish many-septate hyphae in older portions of the mat"; (b) setal hyphae, with walls and contents dark brown, occasionally septate, pointed, of varying lengths, 1.5–7.5  $\mu$  diameter. *Fruit body*: (a) hyphae as in aerial mycelium; (b) setae with walls thick, hyaline to brown, the base bulbous and tip pointed, 6.0–9.0  $\mu$  diameter; (c) basidia subglobose to short clavate, with numerous conspicuous oil globules, bearing four sterigmata and spores; (d) basidiospores hyaline to yellowish, one guttulate, ovoid, slightly flattened on one side, 6.0–9.0  $\times$  4.0–4.5  $\mu$ . *Submerged mycelium*: hyphae as in aerial mycelium.

TYPE OF ROT: white rot of broad-leaved trees, usually *Betula* spp.

DESCRIPTIONS OF CULTURAL CHARACTERS: Campbell (42, under Sterile *Fomes*), Campbell and Davidson (44), Cartwright and Findlay (55, 56), Davidson, Campbell, and Blaisdell (64), Findlay (71).

*Poria obliqua* falls alone in the key, being separated from the similar *Polyporus gilvus* by the slower growth rate of *Poria obliqua*. Cultures of *P. obliqua* exhibit a number of distinctive characters, by which they can be readily identified, the brown fruiting surface being especially noteworthy.

***Poria punctata* Fries**

KEY PATTERN: 1 2 1 2 9 2 2 2 3 2 2

## CULTURES EXAMINED:

CANADA.—Quebec: Merrifield's Corners, on *Salix* sp., F1563; Timagami, on *Acer* sp., F1402.

CULTURAL CHARACTERS: (Pl. XV, Fig. 9; Pl. XVI, Figs. 30 to 32).

GROWTH CHARACTERS.—Growth slow, plates covered in five to six weeks. Advancing zone even, white, slightly raised aerial mycelium extending to limit of growth. Mat white at first and border remaining so until mat has covered plate, becoming "pinkish buff" (9.0YR 7.3/4.5), "chamois" (2.0Y 7.5/5.8), and "tawny-olive" (8.0YR 4.8/5.8) after two to three weeks, raised to top of Petri dish in vicinity of inoculum and gradually sloping to level of agar at margin, thick woolly, sometimes with layer of mycelium growing across inside of lid of Petri dish. Reverse unchanged. No odor. On gallic acid agar diffusion zones very strong, no growth; on tannic acid agar diffusion zones strong, colony 2.5 cm. diameter.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with conspicuous simple septa, 1.5–4.5 $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone, hyaline to brown, with rare simple septa, but with long fiberlike ends lacking septa and branches, 1.5–2.0 $\mu$  diameter. *Submerged mycelium*: hyphae hyaline, frequently branched, the branches often short and curving, with simple septa, 1.5–3.0 $\mu$  diameter.

TYPE OF ROT: white rot of broad-leaved trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64).

In the key, *Poria punctata* falls along with *Polyporus dryophilus* var. *vulpinus* and *Poria ferrea*, from which it is readily separated on the basis of its macroscopic appearance. In *Poria punctata* the mat becomes thicker with increasing age, being finally grown to top of Petri dish and frequently across inner surface of the lid of the Petri dish; in *Polyporus dryophilus* var. *vulpinus* the newest growth is most raised, the older portions of the mat becoming collapsed and felty; in *Poria ferrea*, the mat is all appressed. In addition, *Polyporus dryophilus* var. *vulpinus* occurs on *Populus* spp. only, while the other species occur on a variety of broad-leaved trees.

***Poria rufa* (Schröd. ex Fries) Cooke**

*Poria taxicola* (Pers.) Bres.

KEY PATTERN: 2 1 2 4 9 2 2 2 1 2 2

## CULTURES EXAMINED:

CANADA.—Quebec: Old Chelsea, on conifer, 11252. British Columbia: Aleza Lake, on *Abies lasiocarpa*, 16564, 16615, 16616, on *Picea glauca*, 16560, 16617.

CULTURAL CHARACTERS: (Pl. XV, Fig. 10; Pl. XVI, Figs. 33 and 34).

GROWTH CHARACTERS.—Growth rapid, plates covered in two weeks. Advancing zone even, slightly raised aerial mycelium extending to limit of growth. Mat white, the newest growth raised, cottony-floccose, becoming collapsed, thin downy, the aerial mycelium almost lacking in some isolates, with overgrowth of small compact balls or tufts in certain sectors. Reverse unchanged. No odor. On gallic and tannic acid agars no diffusion zones, colony 1.0–4.0 cm. diameter on gallic acid agar, no growth on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with walls so thin and fragile that the hyphae become twisted and distorted when mounted in potassium hydroxide solution, with occasional single or double clamp connections and inconspicuous simple septa, frequently

branched, 3.0–7.5  $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone, with clamp connections rare, 2.2–4.5 (–6.0)  $\mu$  diameter. *Submerged mycelium*: hyphae as in aerial mycelium, occasionally much-branched to produce a witches' broom effect.

**TYPE OF ROT**: yellow rot of coniferous trees.

*Poria rufa* keys out with *Coniophora puteana* but is readily separated from that species by its scantier production of aerial mycelium, its fragile hyphae with rare, inconspicuous, multiple clamp connections in contrast with the large whorls of clamp connections noticeable on the hyphae of the advancing zone of *Coniophora puteana*, and its association with a white or pale yellow rot while that caused by *C. puteana* is a brown cubical rot.

***Poria subacida*** (Peck) Sacc.

**KEY PATTERN**: (1, 2) 1 1 1 9 2 2 2 (1, 2) 2 (2, 3)

**CULTURES EXAMINED**:

CANADA.—Quebec: Champlain County, on *Picea glauca*, 10258, 10259; Lac Humqui, on *Abies balsamea*, F308; Maniwaki, on *Picea mariana*, 9341, 9770, 9771. Ontario: Timagami, on *Abies balsamea*, F598. British Columbia: Cowichan Lake, on *Thuja plicata*, 11690, 11698; Mission, on *T. plicata*, 9334; Queen Charlotte Islands, on *Picea sitchensis*, 10720, 10737, 11715, on *Tsuga heterophylla*, 10721; Saanichton, on *Pseudotsuga taxifolia*, 9762. UNITED STATES.—Alaska, on *Picea sitchensis*, 11687.

**CULTURAL CHARACTERS**: (Pl. XV, Fig. 11; Pl. XVI, Figs. 35 and 36).

**GROWTH CHARACTERS**.—Growth moderately rapid, plates covered in two to three weeks. Advancing zone even, appressed and hyaline in narrow zone, or with slightly raised aerial mycelium extending to limit of growth. Mat white, in newer growth slightly raised, thin woolly, in area around inoculum appressed, so thin as to be translucent, later raised, uniformly cottony-woolly to felty over whole surface, sometimes grown to top of Petri dish at edge, frequently with minute papillae where mat is grown against side of Petri dish. Reverse unchanged or bleached after five to six weeks. Odor none. On gallic acid agar diffusion zones moderately strong to strong, trace of growth; on tannic acid agar diffusion zones weak to moderately strong, up to 3.0 cm. diameter.

**HYPHAL CHARACTERS**.—*Advancing zone*: hyphae hyaline, nodose-septate, 2.2–5.0  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) fiber hyphae numerous, walls thick and refractive, lumina narrow or apparently lacking, rarely branched, aseptate, long, curving, closely interwoven, 1.5–3.0  $\mu$  diameter. *Submerged mycelium*: (a) hyphae as in advancing zone, frequently branched; (b) crystals numerous, octahedral.

**TYPE OF ROT**: white rot (feather rot, spongy root rot, stringy butt rot) of coniferous and broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS**: Davidson, Campbell, and Blaisdell (64), Fritz (74), Humphrey and Siggers (92).

In the present study, cultures of *Poria subacida* and *P. colorea* appeared to be identical in their cultural characters, so that it was not possible to separate the species. The key patterns for *P. subacida* coincide with those for several other species, most of which lack distinctive diagnostic characters, so that it is difficult to find bases for descriptive keys and positive identifications. *Poria subacida* cultures resemble those of *Fomes pinicola* in their lack of distinctive markings, and this lack, together with the positive reaction on gallic and tannic acid agars, makes it possible to recognize cultures of *Poria subacida* when one has become familiar with them.

***Poria taxicola* (Pers.) Fries**

See *Poria rufa*.

***Poria tsugina* (Murr.) Sacc. and Trott.**

KEY PATTERN: 2 2 1 2 (6,9) 2 2 2 4 2 1

**CULTURES EXAMINED:**

CANADA.—Quebec: Old Chelsea, on *Tsuga canadensis*, 11598. British Columbia: Oyster River, on *Tsuga heterophylla*, 8445; Shaw Creek, on *T. heterophylla*, 8219; Vancouver, on *T. heterophylla*, 8744. UNITED STATES.—Pennsylvania: Cooke Forest, on *Tsuga* sp., F1702.

CULTURAL CHARACTERS: (Pl. XV, Fig. 12; Pl. XVI, Figs. 37 to 39).

GROWTH CHARACTERS.—Growth very slow, radius 3.5–7.5 cm. in six weeks. Two different types of growth may occur in different isolates, in the same isolate in successive plantings, or in different sectors or zones of a colony.—(a) Advancing zone even, with aerial mycelium uniform to limit of growth. Mat white at margin, changing to "cream color" (3.0YR 8.6/4.5), "straw yellow" (5.0YR 9.0/5.5), "honey yellow" (2.0YR 6.7/6.2), "tawny-olive" (8.0YR 4.8/5.8), the newest growth slightly raised, short cottony, becoming opaque woolly, tufted, with brittle, crustose areas "Saccardo's umber" (9.0YR 3.8/3.5) in color formed after three to six weeks, the growth of the aerial mycelium apparently inhibited after five to six weeks. Reverse "buckthorn brown" (8.0YR 4.8/6.5), "cinnamon-brown" (5.0YR 3.0/3.0), "mummy brown" (7.5YR 2.5/2.3), after five to six weeks the color in agar extending beyond aerial mycelium, and apparently being correlated with inhibition of growth. (b) Advancing zone even, appressed, hyaline mycelium over dark brown diffusion zone that extends to limit of growth or beyond. Mat "yellow ochre" (10.0YR 6.8/9.0) to "antique brown" (8.0YR 4.2/5.5), thin cottony, so sparse as to allow darker color of agar to show through and mask color of mat. Reverse "buckthorn brown" (8.0YR 4.8/6.5), "cinnamon-brown" (5.0YR 3.0/3.0), "mummy brown" (7.5YR 2.5/2.3). No odor. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, no growth or a trace on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, thin-walled, with simple septa, frequently branched, 1.5–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, hyaline to pale yellow or dark brown, with walls slightly thickened, branched, 3.0–4.5  $\mu$ , diameter; (b) brown hyphae with walls somewhat thickened, with rare, simple septa, branched but with long unbranched fiberlike ends, 1.0–2.2  $\mu$  diameter; (c) brown hyphae with clublike or knoblike projections, firmly interlocked to form a pseudoparenchymatous crustose layer. *Submerged mycelium*: hyphae as described in (a) above, becoming paler with increasing depth in agar.

TYPE OF ROT: white rot of coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Campbell (42, under *Fomes robustus* var. *tsugina*), Davidson, Campbell, and Blaisdell (64, under *F. robustus* var. *tsugina*).

If the characteristic hyphae making up the pseudoparenchymatous layer are observed and included in the key pattern, then *Poria tsugina* stands alone in the key and should be readily identified. If these special structures are omitted from the key pattern, it falls along with *Fomes nigrolimitatus* and *Polyporus dryadeus*. A descriptive key, which notes general appearance and distribution, has been included to facilitate the separation of these species.

***Poria Vaillantii* (Fries) Cooke**

KEY PATTERN: 2 1 2 1 9 2 2 2 2 (1,2) 2

**CULTURES EXAMINED:**

CANADA.—Quebec: Foster, on beam in house, F5946. Locality and host not known, received from Forest Products Research Laboratory, Princes

Risborough, England, 10444; received from Division of Forest Pathology, United States Department of Agriculture, 11740; received from Bureau voor Schimmelcultures, Baarn, F7319.

**CULTURAL CHARACTERS:** (Pl. XV, Fig. 13; Pl. XVI, Figs. 40 to 42).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, raised aerial mycelium extending to limit of growth. Mat white, slightly raised, cottony-woolly, frequently with strands originating at inoculum and extending across surface or around edge of plate and expanding in plumelike structures, usually bearing fragile, pored fruit bodies after three to four weeks. Reverse unchanged. Odor slight. On gallic and tannic acid agars no diffusion zones, colony 3.0–6.0 cm. diameter on gallic acid agar, no growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 2.2–6.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae numerous in strands and fruiting surface, with walls thick and refractive, lumina apparently lacking, unbranched, aseptate, 2.2–3.0  $\mu$  diameter. *Fruit body:* (a) nodose-septate hyphae and (b) fiber hyphae as described above; (c) basidia 6.0–7.5  $\mu$  diameter, bearing four spores; (d) basidiospores hyaline, even, oblong-ellipsoid, slightly flattened on one side, usually with one large conspicuous oil drop, 4.5–6.0  $\times$  3.0–3.5  $\mu$ . *Submerged mycelium:* (a) hyphae as in advancing zone; (b) crystals numerous, octahedral.

**TYPE OF ROT:** brown cubical rot of coniferous trees, usually on “boards and other forms of structural timber in damp situations”.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Cartwright and Findlay (51, 52, 54, 56), Davidson, Campbell, and Blaisdell (64), Walek-Czernecka (150).

Cartwright and Findlay (54) discussed the “confusion about the identity of this and other related species of *Poria*” and concluded that “In view of the fact that a number of different plants have been included under the name *Poria vaporaria*, it has been decided to apply the name *P. vaillantii* to the fungus which is a frequent cause of dry rot in Great Britain.” The cultures used in the present study include one received from Dr. Cartwright of the Forest Products Research Laboratory in England, an American isolate received from Mr. R. W. Davidson of the Division of Forest Pathology of the United States Department of Agriculture, and other isolates from Ottawa. These are all identical, which indicates that the American concept of the species agrees with that adopted by Cartwright and Findlay.

The key patterns for *P. Vaillantii* coincide with those for several other species, but the unusual appearance of the cultures, with their plumelike growth, noted in the descriptive key, allows for separation. In addition, cultures of this species will be encountered most frequently as isolates from decay in wood in service.

### ***Poria Weirli* Murr.**

**KEY PATTERN:** 2 2 1 2 2 2 2 2 2 (2,3)

#### **CULTURES EXAMINED:**

**CANADA.**—British Columbia: Cowichan Lake, on *Pseudotsuga taxifolia*, F1277, 8186, 8221, 8231, 8470, 9269, 9316, 9323, 9422, 9278, on *Tsuga heterophylla*, 8205, 8288; Lumby, on *Thuja plicata*, 8734. **UNITED STATES.**—Locality not known, on *T. plicata*, 8754.

**CULTURAL CHARACTERS:** (Pl. XV, Fig. 14; Pl. XVI, Figs. 43 to 45).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three weeks. Advancing zone even, sharply delimited, raised aerial mycelium extending to limit of growth. Mat white at first, changing to "tulleul-buff" (9.0YR8.7/1.5), "vinaceous-buff" (7.0YR7.0/3.5), "avellaneous" (8.0YR6.2/3.5), and "wood brown" (7.0YR5.7/4.0), or "cream-buff" (3.0Y8.3/4.5) and "chamois" (2.0Y7.5/5.8) (two weeks), in some isolates becoming "honey yellow" (2.0Y6.7/6.2) and "tawny-olive" (8.0YR4.8/5.8) (four weeks), the color evenly distributed or limited to certain zones, slightly raised, cottony in newest growth, becoming somewhat flattened and woolly, in some isolates producing small areas crustose in texture and "tawny-olive" (8.0YR4.8/5.8) to "Saccardo's umber" (9.0YR3.8/3.5) in color. Reverse unchanged or bleached after three to four weeks. Odor strong and unpleasant at first, but no longer perceptible after four to five weeks. On gallic and tannic acid agars diffusion zones moderately strong, colonies 2.0–4.5 cm. diameter on both media.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, with simple septa, branched, 2.2–6.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) hyphae yellow to brown in potassium hydroxide solution, with walls slightly thickened, frequently septate, branched, 2.2–4.5  $\mu$  diameter, frequently ending in setal hyphae; (c) setal hyphae numerous in all parts of mat, slender, tapering to a point, with walls thick and dark brown, 4.5–6.0 (–7.5)  $\mu$  diameter, up to 350  $\mu$  long. *Submerged mycelium:* (a) hyphae as in advancing zone; (b) crystals numerous, octahedral.

**TYPE OF ROT:** yellow ring rot of coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson, Campbell, and Blaisdell (64), Mounce, Bier, and Nobles (112).

*Poria Weirii* stands alone in the key and so should be readily identifiable. To date it has been reported only from Idaho, Oregon, Washington, and British Columbia, where it has been found causing a laminate butt and root rot of several species of coniferous trees.

***Poria xantha* (Fries) Cooke**

**KEY PATTERN:** (1,2) 1 2 1 9 (1,2) 2 2 2 (1,2) 2

**CULTURES EXAMINED:**

**CANADA.**—British Columbia: Aleza Lake, on *Picea glauca*, 16630, 16645, 16647, on conifer, 16629; Blue River, on *Tsuga heterophylla*, 16088; Cowichan Lake, on *Pseudotsuga taxifolia*, 9315; Prince George, on *Abies* sp., 16570, 16571; Queen Charlotte Islands, on *Picea sitchensis*, 16029; Sooke, on *Tsuga heterophylla*, 16089.

**CULTURAL CHARACTERS:** (Pl. XV, Fig. 15; Pl. XVI, Figs. 46 to 48).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three to four weeks. Advancing zone even, raised, scattered cottony fibers to limit of growth. Mat white, the newest growth raised, loosely arranged, cottony, then abruptly collapsed, aerial mycelium lacking, leaving shining surface, or limited to thin translucent film, sometimes with patches of cottony mycelium scattered over surface, and usually with cottony mycelium grown against walls of Petri dish remote from inoculum to top of dish, in some isolates forming minutely pored fruiting surfaces after five to six weeks. Reverse unchanged. No odor. On gallic and tannic acid agars no diffusion zones (may be slight browning below inoculum), colony 2.0–6.0 cm. diameter on gallic acid agar, no growth or a trace on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 3.0–6.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, frequently with walls or small parts of wall thickened and refractive, often broken into short lengths; (b) nonstaining segments of nodose-septate hyphae, with walls evenly thickened but lumina still visible, 1.2–3.1  $\mu$  diameter; (c) chlamydospores rare or apparently lacking, usually intercalary, rarely terminal, with walls slightly thickened, 10.5–19.5  $\times$  6.0–7.5  $\mu$ . *Fruit body:* (a) basidia 3.7  $\mu$  diameter, bearing four spores; (b) basidiospores hyaline, even, cylindric, slightly curved, 4.2–5.0  $\times$  1.2  $\mu$ . *Submerged mycelium:* hyphae as in aerial mycelium.

**TYPE OF ROT:** brown cubical rot of coniferous and broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Cartwright and Findlay (56), Davidson and Campbell (63), Humphrey and Siggers (92).

Each of the eight key patterns for *Poria xantha* falls with a group of species with identical key patterns. In the inserted descriptive keys separations are based, in the main, on the topography and color of mats. The appearance of cultures of *P. xantha* is characteristic, the surface of older cultures appearing glazed, with aerial mycelium piled up at the edge of the Petri dish and frequently bearing fruiting surfaces.

### **Schizophyllum commune** Fries

**KEY PATTERN:** 1 1 (1,2) 1 9 (1,2) 2 2 2 (1,2) 2

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Wakefield, 11795. Ontario: Ottawa, 11790. Locality not known, on *Betula* sp., F2327. UNITED STATES.—Locality and host not known, F2961.

**CULTURAL CHARACTERS:** (Pl. XVII, Fig. 1; Pl. XVIII, Figs. 1 to 4).

**GROWTH CHARACTERS.**—Growth moderately rapid, plates covered in three weeks. Advancing zone even, except for fans of mycelium extending along edge of Petri dish in advance of periphery of colony and then growing in to meet advancing margin, raised aerial mycelium uniform to limit of growth. Mat white, raised, woolly, frequently grown against wall of Petri dish and down between bottom and lid of dish, with scattered, more or less compact, raised lumps appearing after two to three weeks, some of which develop into fruit bodies that may be normal, i.e. similar to those produced in nature, or much distorted. Reverse unchanged. Odor lacking in young cultures, like honey in six-weeks-old cultures. On gallic acid agar no diffusion zones, on tannic acid agar diffusion zones weak, colonies 3.5–4.4 cm. diameter on both media.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, the cells short, the clamp connections lying close together, 2.2–4.5  $\mu$  diameter. *Aerial mycelium:* (tough, coherent) (a) hyphae as in advancing zone, occasionally with walls somewhat thickened and hyphae then nonstaining, 1.5–6.0  $\mu$  diameter; (b) hyphae as in advancing zone but with numerous minute projections at right angles to cell wall, 2.2–4.5  $\mu$  diameter; (c) chlamydospores fairly numerous in some isolates, rare or apparently lacking in others, terminal and intercalary, with walls slightly thickened, 6.0–15.0  $\times$  4.5–7.5  $\mu$ . *Submerged mycelium:* (a) nodose-septate hyphae and (b) chlamydospores as in aerial mycelium.

**TYPE OF ROT:** white sap rot of broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Cartwright and Findlay (52), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Vaughn (67), Humphrey and Siggers (92), Putterill (127).

Cultures of *Schizophyllum commune* are unusual in that they consistently produce no diffusion zones on gallic acid agar but give a positive reaction on tannic acid agar. This necessitates the inclusion of the species in the key in the group of fungi showing positive reaction on these media and also in the group of species having no diffusion zones. Cultures occasionally fail to form chlamydospores and also to produce fruit bodies within the six weeks' period during which they are under observation. Hence the species appears in the key as having and also as lacking chlamydospores, as fruiting and not fruiting. Each of its eight key patterns coincides with that of one or more other species but identification can be made readily since *S. commune* is

unique in its reaction on gallic and tannic acid agars and in the production of characteristic hyphae having minute projections. Davidson, Campbell, and Vaughn (67) describe these as "short granular side branches" but refer to Vanin who considered them to be "granular crystals". Careful observation leads to the conclusion that the walls of the projections are continuous with the cell wall and that they are in fact "side branches".

### ***Stereum abietinum* Pers.**

KEY PATTERN: 2 2 2 3 9 1 2 2 4 2 1

#### **CULTURES EXAMINED:**

CANADA.—British Columbia: Alberni, on coniferous tree, 16603, 16604; Queen Charlotte Islands, on *Picea sitchensis*, 16090, 16092, on *Tsuga heterophylla*, 16091. UNITED STATES.—Locality and host not known, 11758, 11792.

CULTURAL CHARACTERS: (Pl. XVII, Fig. 2; Pl. XVIII, Figs. 5 to 8).

GROWTH CHARACTERS.—Growth very slow, radius 6.0–7.7 cm. in six weeks. Advancing zone even, appressed and hyaline so that it is difficult to see limit of growth. Outer zone about 1 cm. broad, hyaline, with scanty appressed film of mycelium over agar, next zone "cinnamon-buff" (9.0YR6.6/5.8) and "tawny-olive" (8.0YR4.8/5.8) with aerial mycelium almost completely lacking except for "tawny-olive" woolly mycelium over inoculum. Reverse unchanged below newest growth, then "cinnamon-brown" (5.0YR3.0/3.0), "Mars brown" (4.5YR2.8/3.0), with numerous, conspicuous, opaque, dark brown dots at all levels within agar. Odor lacking in young cultures, penetrating and disagreeable in five- to six-weeks-old cultures. On gallic and tannic acid agars no diffusion zones (agar may become brown below inoculum as on malt agar), no growth or only a trace.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, with rare, inconspicuous, simple septa, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (scanty, mounts scraped from surface) (a) hyphae usually hyaline, rarely with golden brown or dark brown contents, most septa with clamp connections although simple septa, incomplete clamp connections, and normal clamp connections may occur in the same hypha, 2.2–4.5  $\mu$  diameter; (b) hyphae with walls thickened and yellow, lumina narrow but broadening at tip, which may show one or two constrictions, these usually being terminal cells of hyphae as described in (a); (c) chlamydospores numerous, usually terminal but occasionally intercalary, contents hyaline to golden brown, walls at first thin, finally fairly thick, globose to subglobose, 9.0–12.0  $\times$  9.0–10.5  $\mu$ . *Submerged mycelium*: (a) hyaline hyphae and (b) chlamydospores as described above.

TYPE OF ROT: brown cubical rot of coniferous trees.

When one has become familiar with cultures of *Stereum abietinum*, they can be recognized at sight by the characteristic opaque brown dots within the agar, conspicuous in colonies growing in Petri dishes and in culture tubes. In addition, the unusual combination of characters of a brown culture having a negative reaction on gallic and tannic acid agars, and the distinctive hyphae, make cultures of the species easy to key out and identify.

### ***Stereum Murrail* (Berk. and Curt.) Burt**

KEY PATTERN: 1 1 1 1 9 2 2 2 4 2 2

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Gatineau Park, on *Betula papyrifera*, 11362; Mt. Burnet, on *Ostrya virginiana*, F3463.



**CULTURAL CHARACTERS:** (Pl. XVII, Fig. 3; Pl. XVIII, Figs. 9 and 10).

**GROWTH CHARACTERS.**—Growth very slow, radius 2.6–4.0 cm. in six weeks. Advancing zone even, sharply delimited, slightly raised aerial mycelium extending to limit of growth. Mat white, slightly raised; aerial mycelium at first short cottony, later furlike, i.e., fibers short, straight, lying parallel, having a combed appearance, velvety over oldest part, somewhat zonate but thick enough in all parts to be opaque. Reverse unchanged. Odor fairly strong, suggesting rotting apples. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace to 1.5 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 1.5–3.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone; (b) fiber hyphae fairly numerous, frequently branched, aseptate, with walls thick and refractive, 1.0–1.5  $\mu$  diameter. *Submerged mycelium:* hyphae as in advancing zone.

**TYPE OF ROT:** white rot and cankers of broad-leaved tree.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Davidson, Campbell, and Blaisdell (64), Davidson, Campbell, and Lorenz (65).

In the key, *Stereum Murrayi* coincides with only one other species, *Fomes ohiensis*, their slow rate of growth separating them from all other species with similar characteristics. Macroscopically, cultures of *F. ohiensis* and *Stereum Murrayi* are different, the former being appressed, farinaceous, with dots of more compact mycelium, the latter being slightly raised, furry or velvety, all compactly arranged and opaque.

***Stereum sanguinolentum* Alb. and Schw. ex Fries**

**KEY PATTERN:** 2 1 1 4 (1,9) 2 2 2 (2,3) 2 2

**CULTURES EXAMINED:**

CANADA.—Quebec: Champlain County, on *Picea mariana*, 10277; Lac Vert, on *P. mariana*, 10286, 10287, 10288; Mt. Burnet, on coniferous wood, F6510. British Columbia: Aleza Lake, on *Abies* sp., 16587, 16588, 16618; Cowichan Lake, on *Pseudotsuga taxifolia*, 9276; Green Timbers, on *Pinus ponderosa*, 9330; Hidden Lake, on *Thuja plicata*, 16665; Vancouver, on conifer, 9504.

**CULTURAL CHARACTERS:** (Pl. XVII, Fig. 4; Pl. XVIII, Figs. 11 to 14).

**GROWTH CHARACTERS.**—Growth moderately rapid to slow, plates covered in three to five weeks. Advancing zone even or with broad, shallow bays, with sparse, cottony mycelium extending to limit of growth. Mat white at first and remaining so or becoming "cream color" (3.0Y8.6/4.5) to "chamois" (2.0Y7.5/5.8) (four to six weeks), slightly raised, the newer growth downy to cottony-floccose, translucent, the older part felty, opaque. Reverse unchanged to about "honey yellow" (2.0Y6.7/6.2). Odor sweet. On gallic and tannic acid agars diffusion zones moderately strong to strong, trace of growth to colony 1.0 cm. diameter on gallic acid agar, trace of growth to colony 2.0 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, the septa usually simple but occasionally with large clamp connections on the broader hyphae, occurring singly or in pairs at a septum, 2.2–6.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae hyaline, with simple septa, frequently branched, 1.5–4.5 (–6.0)  $\mu$  diameter; (b) cystidiumlike structures, probably analogous to 'conducting cells' of fruit bodies, being expanded ends of hyphae, with walls thickened and refractive, contents at first hyaline and staining in phloxine, then dark brown, 4.5–7.5  $\mu$  diameter, plentiful in some isolates, rare or apparently lacking in others. *Submerged mycelium:* (a) hyphae as in aerial mycelium; (b) crystals large, octahedral.

**TYPE OF ROT:** brown heart rot of coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Cartwright and Findlay (54, 56), Fritz (74), Robak (134).

The key patterns for *Stereum sanguinolentum*, showing the presence of multiple clamp connections on hyphae of the advancing zone, occur alone in the key. Hence, if the nature of the septation is observed in an unidentified culture of this species, it should be possible to key it out readily.

### **Trametes americana** Overholts

KEY PATTERN: 2 1 2 (1,2) 9 (1,2) 2 (1,2) (2,3) 2 2

#### **CULTURES EXAMINED:**

CANADA.—Quebec: Eagle Depot, on *Pinus Banksiana*, 9527; Mt. Burnet, on coniferous wood, 11789. British Columbia: Cowichan Lake, on *Pseudotsuga taxifolia*, 8216. Locality and host not known, 8189, 8190, 9414.

CULTURAL CHARACTERS: (Pl. XVII, Fig. 5; Pl. XVIII, Figs. 15 to 18).

GROWTH CHARACTERS.—Growth moderately rapid to slow, plates covered in four to six weeks. Advancing zone even, hyaline and appressed. Two different types of growth may occur in different isolates, in the same isolate in successive plantings, or in different sectors or zones of a colony: (i) mat white and remaining so or becoming "cream-buff" (3.0Y8.3/4.5), "chamois" (2.0Y7.5/5.8), and "honey yellow" (2.0Y6.7/6.2) over oldest parts, at first short cottony, later woolly, opaque; (ii) mat white, appressed, farinaceous, so thin as to be translucent, this type of mat growing slightly more rapidly than type (i); (iii) mats showing both types of growth, either in sectors, or in zones, the farinaceous type surrounding the cottony-woolly growth. Reverse unchanged. Odor sweet. On gallic and tannic acid agars no diffusion zones, colony up to 1.5 cm. diameter on gallic acid agar, no growth on tannic acid agar.

HYPHAL CHARACTERS.—(Of two types, correlated with the types of growth described above) (i) *Advancing zone*: hyphae hyaline, nodose-septate, frequently branched, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: hyphae as in advancing zone. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores fairly numerous, terminal and intercalary, 9.0–21.0  $\times$  7.5–10.5  $\mu$ . (ii) *Advancing zone*: hyphae hyaline, with simple septa, 1.5–3.0 (–4.5)  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone; (b) oidia numerous, formed by fragmentation of simple septate hyphae, 1.5–3.0  $\mu$  diameter, of varying lengths. *Submerged mycelium*: (a) hyphae as in advancing zone; (b) chlamydospores rare, as described above.

TYPE OF ROT: brown cubical rot of coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64), Mounce and Macrae (113), Snell, Hutchinson, and Newton (140, under *Trametes protracta*).

Cultures of *Trametes americana* exhibit so much variation in macroscopic and microscopic characters as to make their recognition difficult. New isolates have, usually, fairly uniform, cottony to woolly aerial mycelium, composed of nodose-septate hyphae, but they may develop sectors of scanty, farinaceous mycelium made up of hyphae with simple septa, which fragment to form oidia. Cultures that have been in stock for some time and frequently subcultured are all appressed, with farinaceous surface, simple septa, and oidia. Brodie (36) in discussing the records of oidium production in the Hymenomycetes, advanced the suggestion that such oidia "are borne on uninucleate branches arising from the dicaryon mycelium". This seems to be the case in *Trametes americana* where not only do some parts of the mycelium revert to the haploid condition, recognizable by the presence of simple septa and oidia, but these sectors grow more rapidly than the nodose-septate mycelium, so that through successive transfers the nodose-septate mycelium is completely replaced by mycelium with simple septa. A similar change occurs in cultures of *Merulius lacrymans*.

Chlamydospores were observed on the submerged mycelium of all the areas with nodose-septate hyphae, but were rare or lacking in the areas composed of hyphae with simple septa. Therefore the key patterns show chlamydospores with nodose-septate hyphae, but show simple septate hyphae both with and without chlamydospores. Similarly, oidia are always found on hyphae with simple septa, but may or may not be found in a colony having clamp connections, and this correlation is shown in the key. Eight key patterns are necessary for the species, of which five stand alone in the key, the other three coinciding with the key patterns for one or more other species. Descriptive keys are provided for the separation of these groups.

*Trametes americana* has been considered by some authors as a form of *Lenzites saepiaria*. Snell, Hutchinson, and Newton (140) used temperature relations and Mounce and Macrae (113) used interfertility tests to establish the specific rank of each of these species. The present study has shown further marked differences in cultural characters, the most noticeable being the fragmentation of nodose-septate hyphae in *Lenzites saepiaria* to form oidia, and the restriction of oidium formation to hyphae with simple septa in *Trametes americana*.

### **Trametes heteromorpha** (Fries) Bres.

KEY PATTERN: 2 1 2 1 9 2 2 2 3 (1, 2) 2

#### CULTURES EXAMINED:

CANADA.—British Columbia: Aleza Lake, on *Picea glauca*, 16620, on conifer, 16558; Blue River, on *Tsuga heterophylla*, 16094; Hidden Lake, on *T. heterophylla*, 16095; Lumby, on *Picea Engelmanni*, 8440, on *Pseudotsuga taxifolia*, 8439. UNITED STATES.—Pennsylvania: on *Tsuga* sp., 11991.

CULTURAL CHARACTERS: (Pl. XVII, Fig. 7; Pl. XVIII, Figs. 19 to 23).

GROWTH CHARACTERS.—Growth slow, plates covered in five to six weeks. Advancing zone even, appressed, hyaline, the limit of growth usually clearly defined. Mat white, at first downy, translucent, becoming more compact, woolly, opaque, mostly appressed but frequently with a zone of raised mycelium midway across colony, usually fruiting after five to six weeks over area of newest growth remote from inoculum, the fruiting surfaces on slightly raised woolly mycelium, the pores broad with relatively thick dissepiments. Reverse unchanged. Odor slight, fruity. On gallic and tannic acid agars no diffusion zones, colony 2.0–2.5 cm. diameter on gallic acid agar, no growth or only a trace on tannic acid agar.

HYPHAL CHARACTERS.—*Advancing zone*: hyphae hyaline, nodose-septate, frequently branched, 2.2–4.5  $\mu$  diameter. *Aerial mycelium*: (a) hyphae as in advancing zone, up to 6.0  $\mu$  diameter, the broader hyphae frequently having walls irregularly thickened and refractive, and lumina narrow; (b) fiber hyphae numerous, aseptate, rarely branched, 2.2–3.0  $\mu$  diameter, curving and interwoven. *Fruit body*: (a) nodose-septate and (b) fiber hyphae as in aerial mycelium; (c) basidia 6.0–7.5  $\mu$  diameter, bearing four sterigmata up to 7.5  $\mu$  in length; (d) basidiospores hyaline, even, cylindric, slightly flattened on one side, 10.8–13.5  $\times$  3.6–4.5  $\mu$ . *Submerged mycelium*: nodose-septate hyphae as in aerial mycelium.

TYPE OF ROT: brown cubical rot of coniferous trees.

DESCRIPTIONS OF CULTURAL CHARACTERS: Davidson, Campbell, and Blaisdell (64), Mounce (111).

The two key patterns for *Trametes heteromorpha* are identical with those for *T. serialis*, *T. sepium*, and *T. variiformis*. Descriptive keys, in which separations are based on macroscopic appearance and size of basidiospores,

have been inserted but these have not proved satisfactory. It has been found necessary, therefore, to submit isolates that have these key patterns to inter-fertility tests. Such a test requires the isolation of single spore cultures from the unknown culture and the pairing of these with single spore cultures from authentic fruit bodies of *Trametes heteromorpha*, *T. serialis*, and *T. variiformis*. To date it has not been possible to obtain single spore cultures from *T. sepium* and this species has not been included in the tests. To illustrate, V702 was isolated from a decay in *Tsuga heterophylla*. Its cultural characters showed it to belong to one of the species within the group. Pairings were made, and the results are shown in Text-figs. 1, 2, and 3. In these a plus (+) indicates that hyphae bearing clamp connections were formed, a minus (-) indicates that no clamp connections were found on the hyphae in the pairing. It will be

8439					11983						
		1	2	3	6			1	2	3	4
V702	1	+	+	+	+	V702	1	-	-	-	-
	2	+	+	+	+		2	-	-	-	-
	3	+	+	+	+		3	-	-	-	-
	4	+	+	+	+		4	-	-	-	-

TEXT-FIG. 1. Results obtained by pairing four monosporous mycelia from an unidentified culture, V702, with four monosporous mycelia from a fruit body of *Trametes heteromorpha*, 8439.

TEXT-FIG. 2. Results obtained by pairing four monosporous mycelia from an unidentified culture, V702, with four monosporous mycelia from a fruit body of *Trametes serialis*, 11983.

10231					
		3	5	7	8
V702	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-

TEXT-FIG. 3. Results obtained by pairing four monosporous mycelia from an unidentified culture, V702, with four monosporous mycelia from a fruit body of *Trametes variiformis*, 10231.

observed that every pairing between the unknown and *T. heteromorpha* produced mycelium bearing clamp connections, while no clamp connections were produced in the pairings between the unknown fungus and *T. serialis* and *T. variiformis*. Hence the unknown fungus is *T. heteromorpha*. Recourse must be made to interfertility tests for determinations within this group of species until a satisfactory basis of separation on cultural characters is established.

**Trametes sepium Berk.**

KEY PATTERN: (1,2) 1 2 1 9 2 2 2 3 (1,2) 2

**CULTURES EXAMINED:**

UNITED STATES.—Virginia, on *Quercus* sp., 11987, 11988, 11989. Maryland: on *Quercus* sp., 11990.

CULTURAL CHARACTERS: (Pl. XVII, Fig. 8; Pl. XVIII, Figs. 24 to 26).

**GROWTH CHARACTERS.**—Growth slow, plates covered in five weeks. Advancing zone even, aerial mycelium uniform to limit of growth. Mat white, at first appressed, downy, all so thin as to be translucent, soon developing zones in which mycelium is slightly raised, woolly, opaque, the surface becoming pebbled and, after four to six weeks, pored in most isolates. Reverse unchanged. Odor faint, fruity. On gallic and tannic acid agars no diffusion zones, colonies 2.0 cm. diameter on gallic acid agar, trace of growth on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 2.2–3.7  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, frequently up to 6.0  $\mu$  diameter, with walls irregularly thickened and refractive, the lumina irregular, with contents staining in phloxine; (b) fiber hyphae numerous, with walls thick and refractive and lumina entirely lacking, rarely branched, curving and interwoven, 2.2–3.0  $\mu$  diameter. *Submerged mycelium:* (a) nodose-septate hyphae as in aerial mycelium.

TYPE OF ROT: brown rot of broad-leaved or, rarely, coniferous trees.

As was stated under *Trametes heteromorpha*, no satisfactory basis for separating the similar cultures of *T. heteromorpha*, *T. serialis*, and *T. variiformis* has been found. Unlike the other species within the group, *T. sepium* has not produced basidiospores in culture although pored fruiting surfaces have developed. It has not been possible, therefore, to apply the interfertility tests for cultures of *T. sepium*. It may be that this lack of spore production in six-weeks-old cultures may permit recognition of cultures of *T. sepium*.

**Trametes serialis Fries**

KEY PATTERN: (1,2) 1 2 1 9 2 2 2 3 1 2

**CULTURES EXAMINED:**

CANADA.—Quebec: Calumet, on coniferous log, 10230; Eagle Depot, on *Picea mariana*, 9519; Gaspé County, on *Picea* sp., F6813. British Columbia: Queen Charlotte Islands, on *Picea sitchensis*, 10727, 11718. UNITED STATES.—Pennsylvania: Kane, on *Tsuga* sp., 10912. NORWAY.—Oslo, on *Picea excelsa*, F7353, on coniferous log, F7399.

CULTURAL CHARACTERS: (Pl. XVII, Fig. 9; Pl. XVIII, Figs. 27 to 31).

**GROWTH CHARACTERS.**—Growth slow, plates covered in five to six weeks. Advancing zone 1.0–2.0 cm. broad, appressed, hyaline, changing abruptly to white mat, appressed, felty, soon pitted and corrugated to form irregularly pored surface (one to three weeks), which finally extends over whole surface of colony. Reverse unchanged. Odor none or slightly fruity. On gallic and tannic acid agars no diffusion zones (may be slight browning around colony on gallic acid agar), colony 1.0–2.0 cm. diameter on gallic acid agar, trace to 1.0 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, 1.5–4.5 (–6.0)  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, frequently with numerous, small, pointed projections in which walls are thickened and refractive; (b) fiber hyphae very numerous, walls thick and refractive, aseptate, occasionally branched, 1.5–3.0  $\mu$  diameter. *Fruit body:* (a) thin-walled and (b) fiber hyphae as in aerial mycelium; (c) basidia 4.5–6.3  $\mu$  diameter, bearing four sterigmata up to 4.5–7.2  $\mu$  in length; (d) basidiospores hyaline, even, cylindric, (5.4–)6.3–8.1  $\times$  2.2–2.7  $\mu$ . *Submerged mycelium:* (a) nodose-septate hyphae as described above; (b) crystals numerous, octahedral or platelike.

**TYPE OF ROT:** brown cubical rot of broad-leaved and coniferous trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Davidson and Campbell (63), Davidson, Campbell, and Blaisdell (64), Mez (106), Nobles (117), Robak (133, 134), Walek-Czernecka (150).

The difficulty of separating the cultures of *Trametes heteromorpha*, *T. sepium*, *T. serialis*, and *T. variiformis* has been discussed under *T. heteromorpha*. The size of basidiospores of *T. serialis* is a precise diagnostic character, and this, together with the macroscopic appearance of the mats produced by this species may be sufficient to separate it from others. However, the use of interfertility tests to corroborate identifications based on cultural characters is recommended.

***Trametes suaveolens* (L.) Fries**

**KEY PATTERNS:** 1 1 1 1 9 1 2 2 (1,2) 2 3

**CULTURES EXAMINED:**

CANADA.—Quebec: Matapedia, on *Salix* sp., F7654. Ontario: Ottawa, on *Salix* sp., F3522, F3523, F5032. Manitoba: Winnipeg, on *Salix* sp., F2350.

**CULTURAL CHARACTERS:** (Pl. XVII, Fig. 10; Pl. XVIII, Figs. 32 to 34).

**GROWTH CHARACTERS.**—Growth rapid to moderately rapid, plates covered in two to three weeks. Advancing zone even, slightly raised aerial mycelium to limit of growth. Mat white, at first slightly raised, fine downy-floccose, becoming woolly to subfelty but with overgrowth of balls of cottony mycelium around edge of plate and scattered more or less continuously over surface. Reverse unchanged for one week, then completely bleached. Odor strong, sweet. On gallic and tannic acid agars diffusion zones strong, no growth on gallic acid agar, trace on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate, the clamp connections frequently large, 2.2–4.5 $\mu$  diameter. *Aerial mycelium:* hyphae as in advancing zone, usually 1.5–3.0 $\mu$  diameter; (b) fiber hyphae fairly numerous, rarely branched, walls thick and refractive and lumina no longer visible, 1.5–2.2 $\mu$  diameter; (c) chlamydospores numerous, thin-walled, terminal and intercalary, long and cylindric or broadly ovoid, 7.5–15.0  $\times$  4.5–6.0 $\mu$ . *Submerged mycelium:* hyphae as in advancing zone.

**TYPE OF ROT:** white heart rot of *Salix* spp. and, occasionally, *Populus* spp.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3), Hirt (85), Vandendries (141).

*Trametes suaveolens* falls with *Polyporus versicolor* and *P. zonatus* in the key and there appears to be no definite character by which they may be separated. The preference of *Trametes suaveolens* for *Salix* spp. and the strong sweet odor of the culture, similar to that produced by sporophores of the fungus, should make possible the recognition of cultures of this species.

***Trametes tenuis* Karst.**

**KEY PATTERN:** (1,2) (1,2) 1 2 9 2 2 2 4 2 2

**CULTURES EXAMINED:**

CANADA.—British Columbia: Cowichan Lake, on *Thuja plicata*, 8208, 8209, 8217, 9265, 9266, 9293; Yellow Point, on *T. plicata*, 8257.

**CULTURAL CHARACTERS:** (Pl. XVII, Fig. 11; Pl. XVIII, Figs. 35 to 37).

**GROWTH CHARACTERS.**—Growth very slow, radius 5.2–7.0 cm. in six weeks. Advancing zone even or slightly bayed, aerial mycelium uniform to limit of growth. Mat white at first, after three to four weeks developing scattered or more or less continuous areas, especially around inoculum, of "cinnamon-buff" (9.0YR6.6/5.8), "clay color" (10.0YR5.8/6.0), and "ochraceous-tawny" (6.0YR4.9/6.3), appressed, farinaceous to thin felty, minutely tufted. Reverse unchanged, or with zone of blue-green color that disappears after four or five weeks. Odor strong and unpleasant. On gallic and tannic acid agars diffusion zones moderately strong to strong, trace of growth to colonies 1.2 cm. diameter on both media.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, thin-walled, with rare simple septa, 1.5–3.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, with occasional swellings up to 13.5  $\mu$  diameter, these soon appearing devoid of contents and non-staining; (b) hyphae with brown walls, fairly rare, usually the ends of hyaline hyphae, with walls thick and lumina visible only in the broader ones, 1.5–3.0  $\mu$  diameter. *Submerged mycelium:* hyaline hyphae as in aerial mycelium.

**TYPE OF ROT:** white rot of coniferous and broad-leaved trees.

**DESCRIPTIONS OF CULTURAL CHARACTERS:** Badcock (3).

Three of the key patterns for *Trametes tenuis* stand alone in the key, the fourth falls with the key pattern for *Fomes connatus*. The appressed, farinaceous to thin felty mats, always showing patches of color, of cultures of *Trametes tenuis* are completely unlike the white mats of *Fomes connatus*. Hence no difficulty should be experienced in distinguishing between these two species.

### **Trametes variliformis** Peck

**KEY PATTERN:** 2 1 2 1 9 2 2 2 (3,4) (1,2) 2

**CULTURES EXAMINED:**

**CANADA.**—Quebec: Gaspé County, on *Picea glauca*, F8046. British Columbia: Blue River, on *Pinus monticola*, 16097, on *Tsuga heterophylla*, 16098; Clearwater, on *Abies lasiocarpa*, 16038, 16099; Prince George, on *Picea glauca*, 16572, 16573, on *Pseudotsuga taxifolia*, 16608.

**CULTURAL CHARACTERS:** (Pl. XVII, Fig. 12; Pl. XVIII, Figs. 38 to 42).

**GROWTH CHARACTERS.**—Growth slow to very slow, plates covered in six weeks or radius 6.0 cm. or more in six weeks. Advancing zone even, hyaline and appressed, with fibers far apart so that it is difficult to see limit of growth. Mat white, the newer growth appressed, downy to fine woolly, developing small dots of more compact mycelium that finally coalesce to form continuous, felty to pellicular surface over older part, which may become covered with pores after four to five weeks in some isolates. Reverse unchanged. Odor strong, unpleasant. On gallic and tannic acid agars no diffusion zones (may be slight browning below inoculum), colonies 1.0–3.0 cm. diameter on gallic acid agar, trace of growth to colonies 1.3 cm. diameter on tannic acid agar.

**HYPHAL CHARACTERS.**—*Advancing zone:* hyphae hyaline, nodose-septate 1.5–3.0  $\mu$  diameter. *Aerial mycelium:* (a) hyphae as in advancing zone, frequently with walls irregularly thickened and refractive, or with small projections along walls or clamp connections so thickened; (b) fiber hyphae numerous, with walls thick and refractive and lumina apparently lacking, occasionally branched, interwoven to form tough pellicle, 2.2–3.0  $\mu$  diameter. *Fruit body:* (a) basidia scattered or in continuous hymenium, 6.0–9.0  $\mu$  diameter, bearing four spores; (b) basidiospores hyaline, even, cylindric, apiculate, 7.2–9.0  $\times$  2.7–3.6  $\mu$ . *Submerged mycelium:* (a) nodose-septate hyphae as in aerial mycelium; (b) crystals large, octahedral, or small, platelike.

**TYPE OF ROT:** light brown cubical rot of coniferous trees.

## DESCRIPTIONS OF CULTURAL CHARACTERS: Mounce (111).

Only one of the four key patterns for *Trametes variiformis* falls alone in the key. One coincides with the key pattern for *Polyporus fragilis*, cultures of which have thin, translucent mats lacking fiber hyphae, quite unlike cultures of *Trametes variiformis*, which have felty mats consisting mainly of fiber hyphae. The two remaining key patterns fall in the group including *T. heteromorpha*, *T. sepium*, and *T. serialis*. The remarks on separations within the group under *T. heteromorpha* are equally applicable to *T. variiformis*.

## Discussion

Following each field season in recent years several hundred cultures of wood-rotting fungi have been identified with the aid of the foregoing key. The majority of these cultures were isolated by Dr. J. E. Bier, formerly of the Dominion Laboratory of Forest Pathology, Victoria, and his associates, in the course of their studies of decay in various species of British Columbia trees. Early in their investigations the difficulty of determining the causal organisms by direct examination of the rots was recognized and "cultures were prepared from all decays that could not be determined positively in the field". These were submitted to the author for identification.

In most projects a large percentage of the cultures, usually about 80%, are readily identifiable but the remaining isolates frequently defy identification. These may be unusual forms of species included in the key but more often belong to species not represented there or in the stock culture collection. Identification must be delayed until named cultures showing identical characters are obtained from fruit bodies. Some of the cultures that proved difficult to determine have been found to belong to undescribed species. Thus *Lentinus Kauffmanii*, of which cultures were obtained from decay in 1925, was described and named only in 1946 (Bier and Nobles (30) ). Other cultures, such as *Omphalia campanella* and *Trechispora raduloides*, remained unidentified until linked with cultures from species previously listed as saprophytes and not suspected of causing decay in living trees. Meanwhile the steadily growing accumulation of unidentified cultures from rots, stresses the necessity for continuous research. Mycological studies, involving the collection and identification of sporophores from the hosts and localities under consideration, and the isolation and description of cultures from them should precede or accompany pathological studies. Only by this means can comprehensive information with regard to the fungi that cause decay be accumulated.

The identification of the majority of the cultures submitted has provided precise data on the species of fungi responsible for decay in the hosts under consideration. This information has been incorporated in a number of publications from the Victoria Laboratory, such as *Decay of Sitka Spruce on the Queen Charlotte Islands*, by Bier, Foster, and Salisbury (29).

These authors reported that "This work demonstrated that overmature Sitka spruce is attacked by many wood-destroying fungi", and supported their



statement with a list of 31 species found causing decay in living Sitka spruce on the Queen Charlotte Islands. Similarly Davidson, Campbell, and Vaughn (67) in their publication *Fungi Causing Decay of Living Oaks in the Eastern United States and Their Cultural Identification* stated "As a result of these studies a number of fungi that were not previously known to cause important heart rot in oaks were obtained", and listed 47 species that had been found to produce decay in that host. Other authors have reported similar observations. Thus the result of the application of the cultural method to decay studies has been to increase the number of species known to be of pathological significance in the various hosts and to emphasize the complex nature of the decay problem. It would appear that the method must be adopted whenever precise information on the causes of decay in a given host is required.

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## Index to Genera and Species

	PAGE		PAGE
<i>abietinus</i> ( <i>Polyporus</i> )	349	<i>dryophilus</i> var. <i>vulpinus</i>	
<i>abietinum</i> ( <i>Stereum</i> )	397	( <i>Polyporus</i> )	363
<i>albipellucida</i> ( <i>Poria</i> )	384	ECHINODONTIUM	317
<i>adiposa</i> ( <i>Pholiota</i> )	347	<i>Ellisianus</i> ( <i>Fomes</i> )	321
<i>adustus</i> ( <i>Polyporus</i> )	350	<i>Everhartii</i> ( <i>Fomes</i> )	322
<i>albellus</i> ( <i>Polyporus</i> )	350	FAVOLUS	318
<i>alveolaris</i> ( <i>Favolus</i> )	318	<i>ferrea</i> ( <i>Poria</i> )	387
<i>americana</i> ( <i>Trametes</i> )	399	<i>ferrugineo-fusca</i> ( <i>Poria</i> )	388
<i>amorphus</i> ( <i>Polyporus</i> )	351	<i>ferruginosa</i> ( <i>Poria</i> )	388
<i>anceps</i> ( <i>Polyporus</i> )	352	<i>fibrillosus</i> ( <i>Polyporus</i> )	364
<i>annosus</i> ( <i>Fomes</i> )	319	<i>fomentarius</i> ( <i>Fomes</i> )	322
<i>applanatum</i> ( <i>Ganoderma</i> )	335	FOMES	319
<i>arcularius</i> ( <i>Polyporus</i> )	352	<i>fragilis</i> ( <i>Polyporus</i> )	364
<i>aurivella</i> ( <i>Pholiota</i> )	347	<i>fraxineus</i> ( <i>Fomes</i> )	323
ARMILLARIA	313	<i>fraxinophilus</i> ( <i>Fomes</i> )	324
<i>asiatica</i> ( <i>Poria</i> )	385	<i>frondosus</i> ( <i>Polyporus</i> )	365
<i>balsameus</i> ( <i>Polyporus</i> )	353	<i>fulvus</i> ( <i>Fomes</i> )	324
<i>Berkeleyi</i> ( <i>Polyporus</i> )	354	<i>fumosus</i> ( <i>Polyporus</i> )	365
<i>betulina</i> ( <i>Lenzites</i> )	342	<i>galactinus</i> ( <i>Polyporus</i> )	366
<i>betulinus</i> ( <i>Polyporus</i> )	354	GANODERMA	335
<i>borealis</i> ( <i>Polyporus</i> )	355	<i>gigantea</i> ( <i>Peniophora</i> )	346
<i>brumalis</i> ( <i>Polyporus</i> )	356	<i>gilvus</i> ( <i>Polyporus</i> )	367
<i>campanella</i> ( <i>Omphalia</i> )	345	<i>glomeratus</i> ( <i>Polyporus</i> )	367
<i>carbonica</i> ( <i>Poria</i> )	386	<i>graveolens</i> ( <i>Polyporus</i> )	368
<i>cinerescens</i> ( <i>Poria</i> )	387	<i>guttulatus</i> ( <i>Polyporus</i> )	369
<i>cinnabarinus</i> ( <i>Polyporus</i> )	356	<i>heteromorpha</i> ( <i>Trametes</i> )	400
<i>circinatus</i> ( <i>Polyporus</i> )	357	<i>hirsutus</i> ( <i>Polyporus</i> )	370
COLLYBIA	313	HYMENOCHAETE	339
<i>colorea</i> ( <i>Poria</i> )	387	<i>igniarius</i> ( <i>Fomes</i> )	325
<i>commune</i> ( <i>Schizophyllum</i> )	396	<i>igniarius</i> var. <i>laevigatus</i> ( <i>Fomes</i> )	326
<i>compactus</i> ( <i>Polyporus</i> )	358	<i>igniarius</i> var. <i>populinus</i> ( <i>Fomes</i> )	327
<i>conchatus</i> ( <i>Fomes</i> )	320	<i>Kauffmanii</i> ( <i>Lentinus</i> )	340
<i>conchifer</i> ( <i>Polyporus</i> )	359	<i>lacrymans</i> ( <i>Merulius</i> )	344
<i>confragosa</i> ( <i>Daedalea</i> )	315	LENTINUS	340
CONIOPHORA	315	LENZITES	342
<i>connatus</i> ( <i>Fomes</i> )	320	<i>lepideus</i> ( <i>Lentinus</i> )	341
<i>corrugata</i> ( <i>Hymenochaete</i> )	339	<i>lobatum</i> ( <i>Ganoderma</i> )	336
<i>cuticularis</i> ( <i>Polyporus</i> )	359	<i>lucidum</i> ( <i>Ganoderma</i> )	336
DAEDALEA	315	<i>mellea</i> ( <i>Armillaria</i> )	313
<i>dichrous</i> ( <i>Polyporus</i> )	360	MERULIUS	344
<i>distortus</i> ( <i>Polyporus</i> )	361	<i>microspora</i> ( <i>Poria</i> )	389
<i>dryadeus</i> ( <i>Polyporus</i> )	361	<i>mollis</i> ( <i>Polyporus</i> )	370
<i>dryophilus</i> ( <i>Polyporus</i> )	362	<i>montanus</i> ( <i>Polyporus</i> )	371

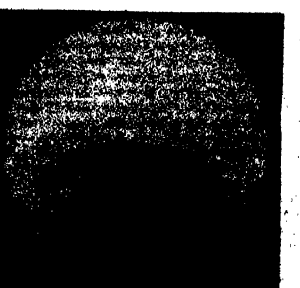
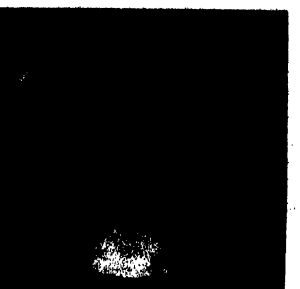
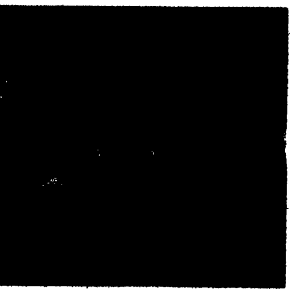
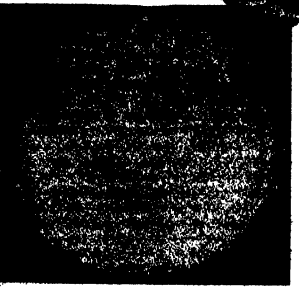
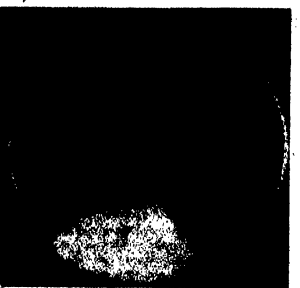
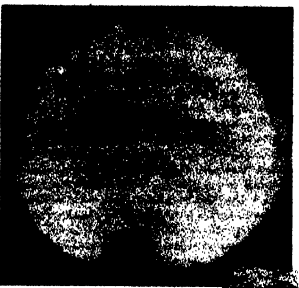
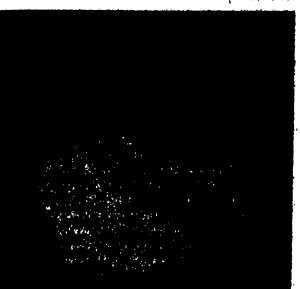
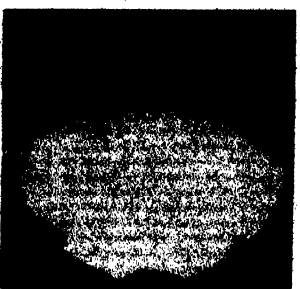
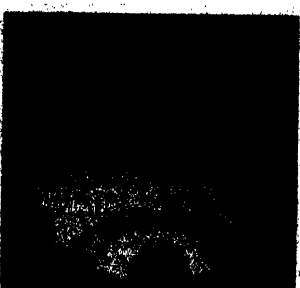
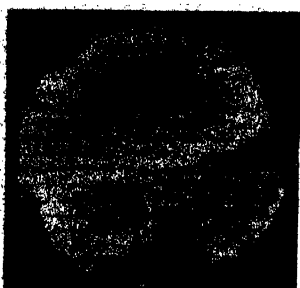
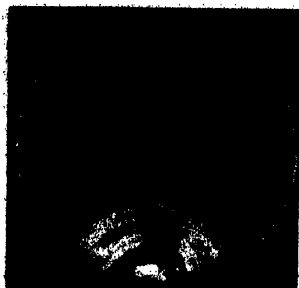
	PAGE		PAGE
<i>monticola</i> ( <i>Poria</i> )	389	SCHIZOPHYLLUM	396
<i>Murratii</i> ( <i>Stereum</i> )	397	<i>Schweinitzii</i> ( <i>Polyporus</i> )	377
<i>nigrolimitatus</i> ( <i>Fomes</i> )	327	<i>scutellatus</i> ( <i>Fomes</i> )	333
<i>obliqua</i> ( <i>Poria</i> )	390	<i>semipileatus</i> ( <i>Polyporus</i> )	378
<i>obtusus</i> ( <i>Polyporus</i> )	372	<i>sepium</i> ( <i>Trametes</i> )	402
<i>officinalis</i> ( <i>Fomes</i> )	328	<i>serialis</i> ( <i>Trametes</i> )	402
<i>ohiensis</i> ( <i>Fomes</i> )	329	<i>squamosus</i> ( <i>Polyporus</i> )	378
OMPHALIA	345	STEREUM	397
<i>oregonense</i> ( <i>Ganoderma</i> )	338	<i>suaveolens</i> ( <i>Trametes</i> )	403
<i>ostreatus</i> ( <i>Pleurotus</i> )	347	<i>subacida</i> ( <i>Poria</i> )	392
<i>palustris</i> ( <i>Polyporus</i> )	373	<i>subcartilagineus</i> ( <i>Polyporus</i> )	379
<i>pargamenus</i> ( <i>Polyporus</i> )	373	<i>subroseus</i> ( <i>Fomes</i> )	334
PENIOPHORA	346	<i>sulphureus</i> ( <i>Polyporus</i> )	380
PHOLIOTA	347	<i>tabacina</i> ( <i>Hymenochaete</i> )	340
<i>Pini</i> ( <i>Fomes</i> )	330	<i>taxicola</i> ( <i>Poria</i> )	393
<i>pinicola</i> ( <i>Fomes</i> )	331	<i>tenuis</i> ( <i>Trametes</i> )	403
PLEUROTUS	347	<i>tigrinus</i> ( <i>Lentinus</i> )	341
POLYPORUS	349	<i>tinctorium</i> ( <i>Echinodontium</i> )	317
<i>pomaceus</i> ( <i>Fomes</i> )	332	<i>tomentosus</i> ( <i>Polyporus</i> )	357
PORIA	384	<i>trabea</i> ( <i>Lenzites</i> )	343
<i>protracta</i> ( <i>Trametes</i> )—		TRAMETES	399
( <i>T. americana</i> )	399	<i>Tsugae</i> ( <i>Ganoderma</i> )	338
<i>pubescens</i> ( <i>Polyporus</i> )	374	<i>tsugina</i> ( <i>Poria</i> )	393
<i>punctata</i> ( <i>Poria</i> )	391	<i>tuberaster</i> ( <i>Polyporus</i> )	380
<i>puteana</i> ( <i>Coniophora</i> )	315	<i>Tulipiferae</i> ( <i>Polyporus</i> )	381
<i>quercina</i> ( <i>Daedalea</i> )	316	<i>ulmarius</i> ( <i>Pleurotus</i> )	348
<i>radiatus</i> ( <i>Polyporus</i> )	375	<i>umbellatus</i> ( <i>Polyporus</i> )	382
<i>radicata</i> ( <i>Collybia</i> )	313	<i>unicolor</i> ( <i>Daedalea</i> )	317
<i>resinosus</i> ( <i>Polyporus</i> )	375	<i>Vaillantii</i> ( <i>Poria</i> )	393
<i>rimosus</i> ( <i>Fomes</i> )	332	<i>variiformis</i> ( <i>Trametes</i> )	404
<i>robustus</i> var. <i>tsugina</i> ( <i>Fomes</i> )	393	<i>velutipes</i> ( <i>Collybia</i> )	314
<i>roseus</i> ( <i>Fomes</i> )	332	<i>versicolor</i> ( <i>Polyporus</i> )	382
<i>rufa</i> ( <i>Poria</i> )	391	<i>volvatus</i> ( <i>Polyporus</i> )	383
<i>rutilans</i> ( <i>Polyporus</i> )	376	<i>Weirii</i> ( <i>Poria</i> )	394
<i>saepiaria</i> ( <i>Lenzites</i> )	343	<i>xantha</i> ( <i>Poria</i> )	395
<i>sanguinolentum</i> ( <i>Stereum</i> )	398	<i>zonatus</i> ( <i>Polyporus</i> )	384



## PLATE I

FIGS. 1 TO 15. *Cultures grown on malt agar in the dark.*

- FIG. 1. *Armillaria mellea*, 8559, *six weeks old.*
- FIG. 2. *Collybia radicata*, F3461, *six weeks old.*
- FIG. 3. *Collybia velutipes*, F1598, *four weeks old.*
- FIG. 4. *Coniophora puteana*, 8547, *two weeks old.*
- FIG. 5. *Daedalea confragosa*, 9210, *two weeks old.*
- FIG. 6. *Daedalea quercina*, F2158, *five weeks old.*
- FIG. 7. *Daedalea unicolor*, F8022, *three weeks old.*
- FIG. 8. *Echinodontium tinctorium*, F1157, *four weeks old.*
- FIG. 9. *Favolus alveolaris*, F1338, *four weeks old.*
- FIG. 10. *Fomes annosus*, 8429, *four weeks old.*
- FIG. 11. *Fomes conchatus*, 9219, *four weeks old.*
- FIG. 12. *Fomes connatus*, F7990, *four weeks old.*
- FIG. 13. *Fomes Ellisianus*, F7520, *four weeks old.*
- FIG. 14. *Fomes Everhartii*, F3580, *four weeks old.*
- FIG. 15. *Fomes fomentarius*, 9214, *four weeks old.*



## PLATE II

FIGS. 1 TO 48. All  $\times 455$ .

FIGS. 1 TO 3. *Armillaria mellea*. FIG. 1. *Hypha from advancing zone*. FIG. 2. *Rough-walled aerial hypha*. FIG. 3. *Cuticular cells*.

FIGS. 4 TO 6. *Collybia radicata*. FIG. 4. *Hypha from advancing zone*. FIG. 5. *Aerial hypha*. FIG. 6. *Submerged hypha*.

FIGS. 7 TO 9. *Collybia velutipes*. FIG. 7. *Hypha from advancing zone*. FIG. 8. *Aerial hypha bearing oidia*. FIG. 9. *Lactiferous cell*.

FIGS. 10 TO 14. *Coniophora cerebella*. FIGS. 10, 11. *Hyphae from advancing zone*. FIG. 10. *Hyphal tips*. FIG. 11. *Multiple clamp connections*. FIG. 12. *Rough-walled brown aerial hypha*. FIG. 13. *Hyaline aerial hypha*. FIG. 14. *Oidia*.

FIGS. 15 TO 17. *Daedalea confragosa*. FIG. 15. *Hypha from advancing zone*. FIG. 16. *Fiber hypha*. FIG. 17. *Much-branched hyphae from skinlike layer*.

FIGS. 18 TO 24. *Daedalea quercina*. FIG. 18. *Hypha from advancing zone*. FIG. 19. *Thick-walled aerial hypha*. FIG. 20. *Fiber hypha*. FIG. 21. *Chlamydospores*. FIG. 22. *Basidia*. FIG. 23. *Basidiospores*. FIG. 24. *Hypha from submerged mycelium*.

FIGS. 25 TO 26. *Daedalea unicolor*. FIG. 25. *Aerial hyphae*. FIG. 26. *Fiber hyphae*.

FIGS. 27 TO 30. *Echinodontium tinctorium*. FIG. 27. *Hypha from advancing zone*. FIG. 28. *Aerial hypha*. FIG. 29. *Chlamydospores*. FIG. 30. *Cystidia*.

FIGS. 31 TO 32. *Favolus alveolaris*. FIG. 31. *Hypha from advancing zone*. FIG. 32. *Fiber hypha*.

FIGS. 33 TO 35. *Fomes annosus*. FIG. 33. *Hyphae from advancing zone*. FIG. 34. *Conidiophores*. FIG. 35. *Conidia*.

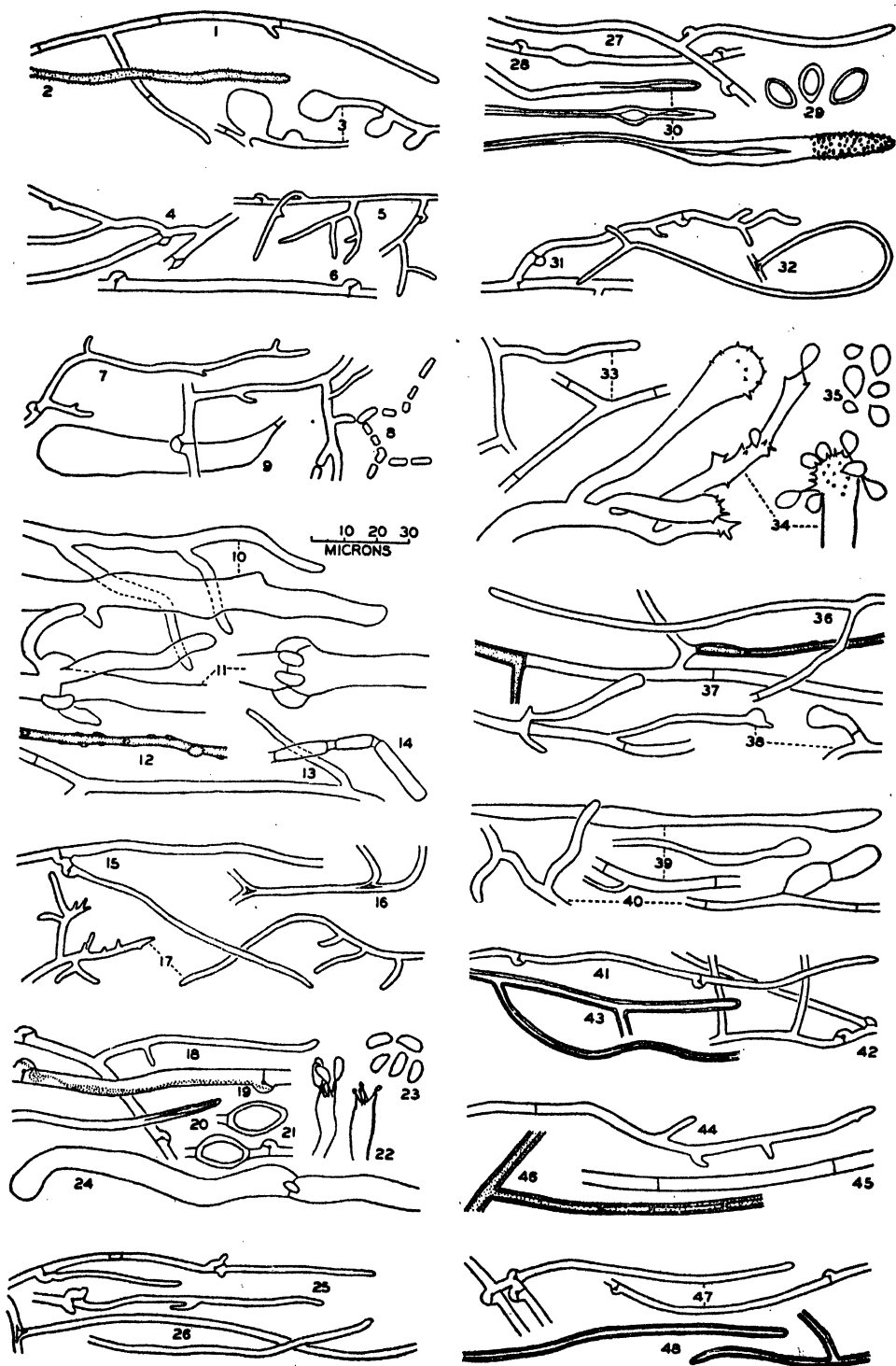
FIGS. 36 TO 38. *Fomes conchatus*. FIG. 36. *Hypha from advancing zone*. FIG. 37. *Partly colored aerial hypha*. FIG. 38. *Submerged hyphae*.

FIGS. 39 TO 40. *Fomes connatus*. FIG. 39. *Aerial hyphae*. FIG. 40. *Submerged hyphae*.

FIGS. 41 TO 43. *Fomes Ellisianus*. FIG. 41. *Hypha from advancing zone*. FIG. 42. *Aerial hypha*. FIG. 43. *Fiber hypha*.

FIGS. 44 TO 46. *Fomes Everhartii*. FIG. 44. *Hypha from advancing zone*. FIG. 45. *Aerial hyphae*. FIG. 46. *Brown aerial hypha*.

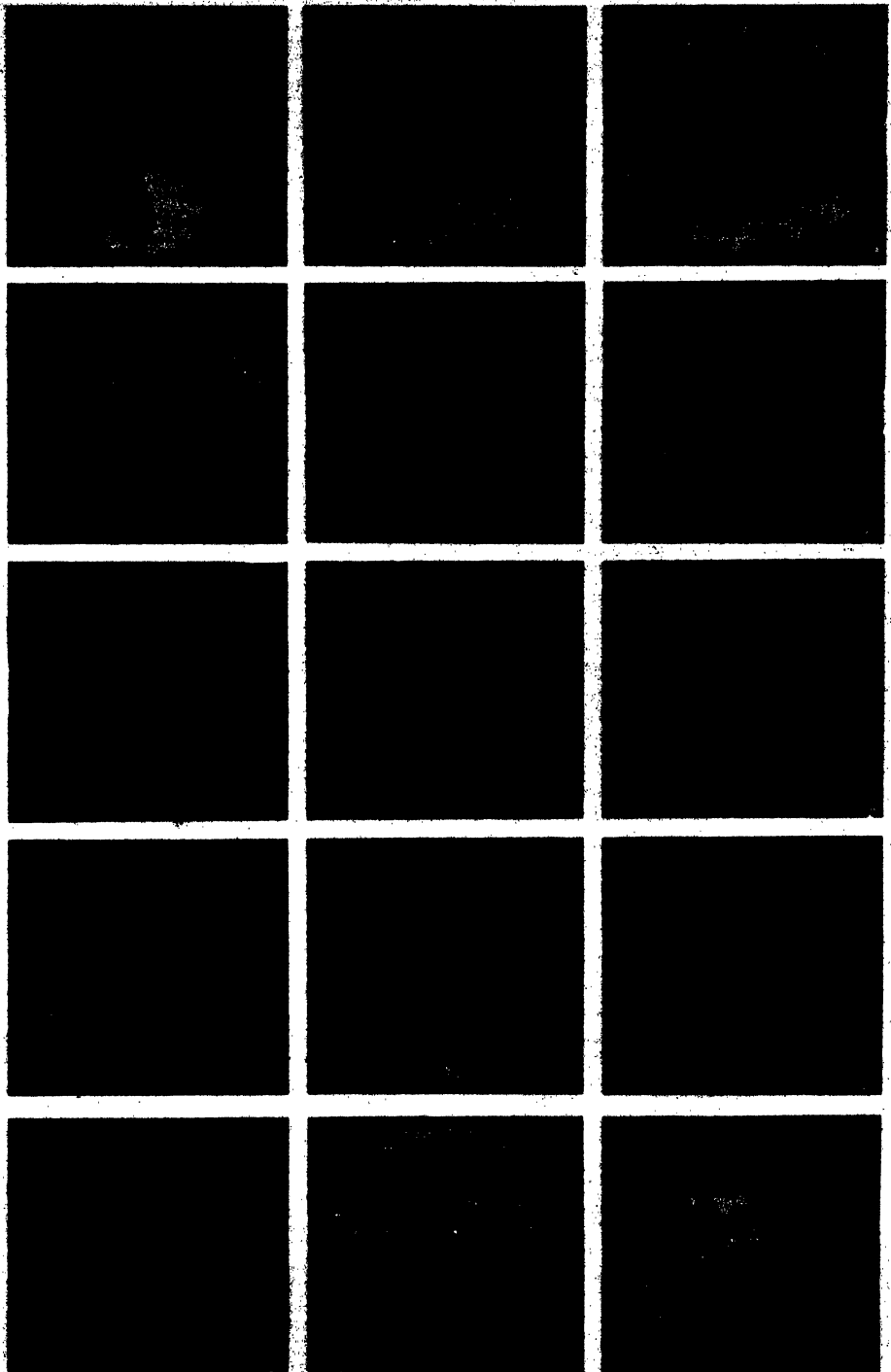
FIGS. 47 TO 48. *Fomes fomentarius*. FIG. 47. *Hyphae from advancing zone*. FIG. 48. *Fiber hyphae*.



## PLATE III

FIGS. 1 TO 15. *Cultures grown on malt agar in the dark.*

- FIG. 1. *Fomes fraxineus*, F7519, *four weeks old.*
- FIG. 2. *Fomes fraxinophilus*, F1932, *four weeks old.*
- FIG. 3. *Fomes fulvus*, F2283, *four weeks old.*
- FIG. 4. *Fomes igniarius*, F7974, *four weeks old.*
- FIG. 5. *Fomes igniarius* var. *laevigatus*, 9008, *four weeks old.*
- FIG. 6. *Fomes igniarius* var. *populinus*, R4464, *four weeks old.*
- FIG. 7. *Fomes nigrolimitatus*, 9159, *six weeks old.*
- FIG. 8. *Fomes officinalis*, 9503, *six weeks old.*
- FIG. 9. *Fomes ohiensis*, F8005, *six weeks old.*
- FIG. 10. *Fomes Pini*, F2366, *six weeks old.*
- FIG. 11. *Fomes pinicola*, 9033, *four weeks old.*
- FIG. 12. *Fomes rimosus*, F2165, *six weeks old.*
- FIG. 13. *Fomes roseus*, F1449, *four weeks old.*
- FIG. 14. *Fomes scutellatus*, F3444, *four weeks old.*
- FIG. 15. *Fomes subroseus*, 8184, *four weeks old.*



## PLATE IV

FIGS. 1 TO 58. All  $\times 455$ .

FIGS. 1 TO 6. *Fomes fraxineus*. FIG. 1. *Hypha from advancing zone*. FIG. 2. *Fiber hypha*. FIG. 3. *Chlamydospores*. FIG. 4. *Staghorn-branched hypha*. FIG. 5. *Basidia*. FIG. 6. *Basidiospores*.

FIGS. 7 TO 11. *Fomes fraxinophilus*. FIG. 7. *Hyphae from advancing zone*. FIG. 8. *Fiber hypha*. FIG. 9. *Chlamydospores*. FIG. 10. *Basidia*. FIG. 11. *Basidiospores*.

FIGS. 12 TO 15. *Fomes fulvus*. FIG. 12. *Hyphae from advancing zone*. FIG. 13. *Brown aerial hyphae*. FIG. 14. *Helicoid aerial hypha*. FIG. 15. *Submerged hypha*.

FIGS. 16 TO 19. *Fomes igniarius*. FIG. 16. *Hyphae from advancing zone*. FIG. 17. *Pale aerial hypha*. FIG. 18. *Brown aerial hypha*. FIG. 19. *Cuticular cells*.

FIGS. 20 TO 22. *Fomes igniarius var. laevigatus*. FIG. 20. *Hypha from advancing zone*. FIG. 21. *Brown aerial hypha*. FIG. 22. *Cuticular cells*.

FIGS. 23 TO 25. *Fomes igniarius var. populinus*. FIG. 23. *Hyphae from advancing zone*. FIG. 24. *Brown aerial hypha*. FIG. 25. *Cuticular cells*.

FIGS. 26 TO 29. *Fomes nigrolimitatus*. FIG. 26. *Hyphae from advancing zone*. FIG. 27. *Aerial hypha*. FIG. 28. *Brown aerial hypha*. FIG. 29. *Bulbils*.

FIGS. 30 TO 33. *Fomes officinalis*. FIG. 30. *Hypha from advancing zone*. FIG. 31. *Conidiophores*. FIG. 32. *Conidia*. FIG. 33. *Chlamydospores*.

FIG. 34. *Fomes ohiensis*. FIG. 34. *Hyphae*.

FIGS. 35 TO 38. *Fomes Pini*. FIG. 35. *Hyphae from advancing zone*. FIG. 36. *Aerial hyphae*. FIG. 37. *Aerial hyphae showing expansions*. FIG. 38. *Setae*.

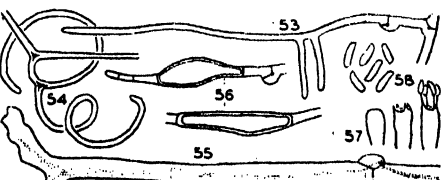
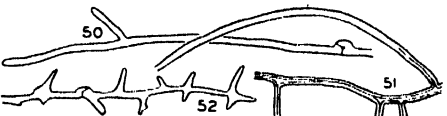
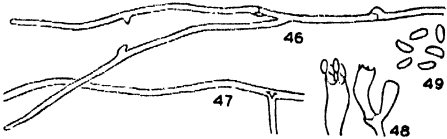
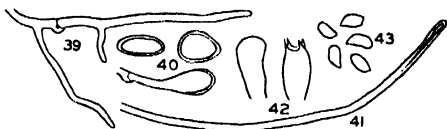
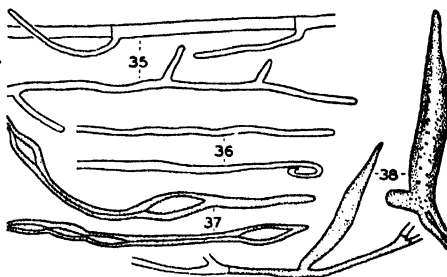
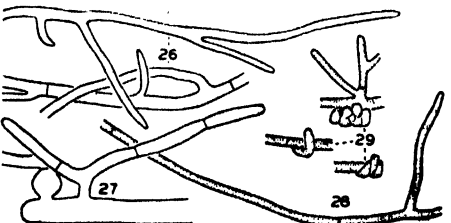
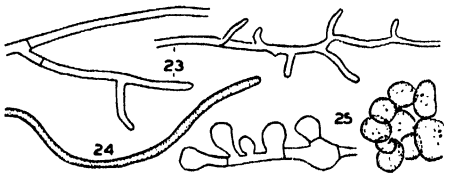
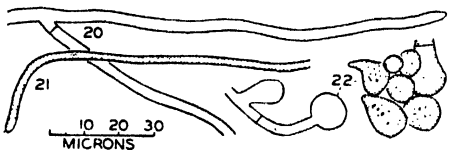
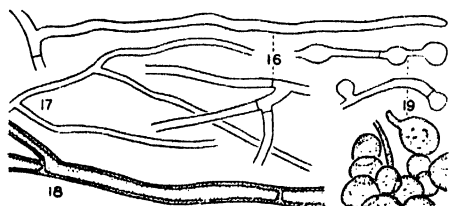
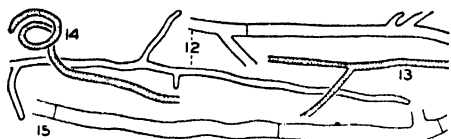
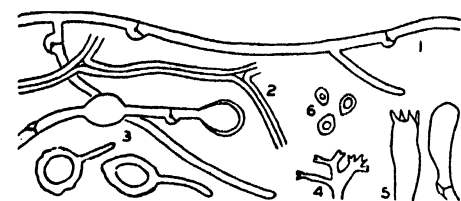
FIGS. 39 TO 43. *Fomes pinicola*. FIG. 39. *Hyphae from advancing zone*. FIG. 40. *Chlamydospores*. FIG. 41. *Fiber hypha*. FIG. 42. *Basidia*. FIG. 43. *Basidiospores (from fruit body produced in culture on elm wood block on prune agar, four months old)*.

FIGS. 44 TO 45. *Fomes rimosus*. FIG. 44. *Hyphae from advancing zone*. FIG. 45. *Brown aerial hyphae*.

FIGS. 46 TO 49. *Fomes roseus*. FIG. 46. *Hypha from advancing zone*. FIG. 47. *Fiber hypha*. FIG. 48. *Basidia*. FIG. 49. *Basidiospores*.

FIGS. 50 TO 52. *Fomes scutellatus*. FIG. 50. *Hypha from advancing zone*. FIG. 51. *Fiber hypha*. FIG. 52. *Submerged hypha*.

FIGS. 53 TO 58. *Fomes subroseus*. FIG. 53. *Hypha from advancing zone*. FIG. 54. *Fiber hypha*. FIG. 55. *Broad aerial hypha*. FIG. 56. *Chlamydospores*. FIG. 57. *Basidia*. FIG. 58. *Basidiospores*.

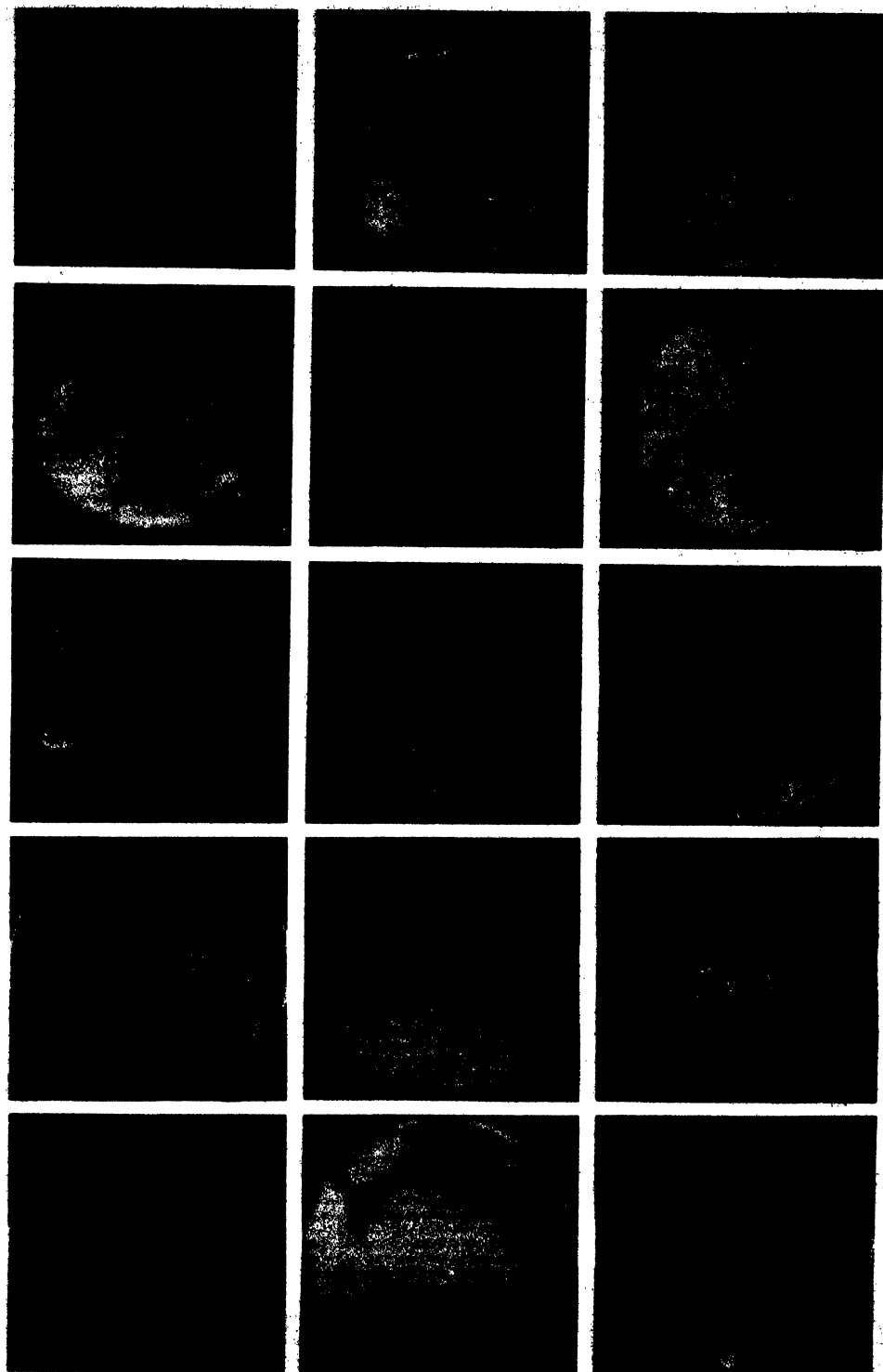




## PLATE V

FIGS. 1 TO 15. *Cultures grown on malt agar in the dark.*

- FIG. 1. *Ganoderma applanatum*, 8180, six weeks old.
- FIG. 2. *Ganoderma lobatum*, F8006, four weeks old.
- FIG. 3. *Ganoderma lucidum*, 10222, six weeks old.
- FIG. 4. *Ganoderma oregonense*, F1274, six weeks old.
- FIG. 5. *Ganoderma Tsugae*, 9346, four weeks old.
- FIG. 6. *Hymenochaete corrugata*, F2940, four weeks old.
- FIG. 7. *Hymenochaete tabacina*, 9290, four weeks old.
- FIG. 8. *Lentinus Kauffmanii*, 10723, six weeks old.
- FIG. 9. *Lentinus lepideus*, F627, four weeks old.
- FIG. 10. *Lentinus tigrinus*, F3048, four weeks old.
- FIG. 11. *Lenzites betulina*, 10199, four weeks old.
- FIG. 12. *Lenzites saepiaria*, 9419, six weeks old.
- FIG. 13. *Lenzites trabea*, F3823, four weeks old.
- FIG. 14. *Merulius lacrymans*, 8787, five weeks old.
- FIG. 15. *Merulius lacrymans*, same isolate as Fig. 14, after reverting to the haploid condition, six weeks old.



## PLATE VI

FIGS. 1 TO 53. All  $\times 455$ .

FIGS. 1 TO 4. *Ganoderma applanatum*. FIG. 1. *Hyphae from advancing zone*. FIG. 2. *Fiber hypha*. FIG. 3. *Staghorn-branched hypha*. FIG. 4. *Cuticular cells*.

FIGS. 5 TO 8. *Ganoderma lobatum*. FIG. 5. *Hypha from advancing zone*. FIG. 6. *Fiber hypha*. FIG. 7. *Staghorn-branched hypha*. FIG. 8. *Cuticular cells*.

FIGS. 9 TO 13. *Ganoderma lucidum*. FIG. 9. *Hypha from advancing zone*. FIG. 10. *Fiber hypha*. FIG. 11. *Staghorn-branched hypha*. FIG. 12. *Chlamydospores*. FIG. 13. *Cuticular cells*.

FIGS. 14 TO 16. *Ganoderma oregonense*. FIG. 14. *Hypha from advancing zone*. FIG. 15. *Aerial hypha*. FIG. 16. *Cuticular cells*.

FIGS. 17 TO 20. *Ganoderma Tsugae*. FIG. 17. *Hypha from advancing zone*. FIG. 18. *Fiber hypha*. FIG. 19. *Staghorn-branched hypha*. FIG. 20. *Cuticular cells*.

FIGS. 21 TO 22. *Hymenochaete corrugata*. FIG. 21. *Hypha from advancing zone*. FIG. 22. *Aerial hypha*.

FIGS. 23 TO 26. *Hymenochaete tabacina*. FIG. 23. *Hypha from advancing zone*. FIG. 24. *Hyaline aerial hypha*. FIG. 25. *Brown aerial hypha*. FIG. 26. *Cells from pseudoparenchymatous layer*.

FIGS. 27 TO 29. *Lentinus Kauffmanii*. FIG. 27. *Hyphae from advancing zone*. FIG. 28. *Aerial hypha*. FIG. 29. *Fiber hypha*.

FIGS. 30 TO 34. *Lentinus lepideus*. FIG. 30. *Hyphae from advancing zone*. FIG. 31. *Aerial hypha*. FIG. 32. *Fiber hypha*. FIG. 33. *Chlamydospores*. FIG. 34. *Swollen submerged hypha*.

FIGS. 35 TO 36. *Lentinus tigrinus*. FIG. 35. *Hyphae from advancing zone*. FIG. 36. *Chlamydospores*.

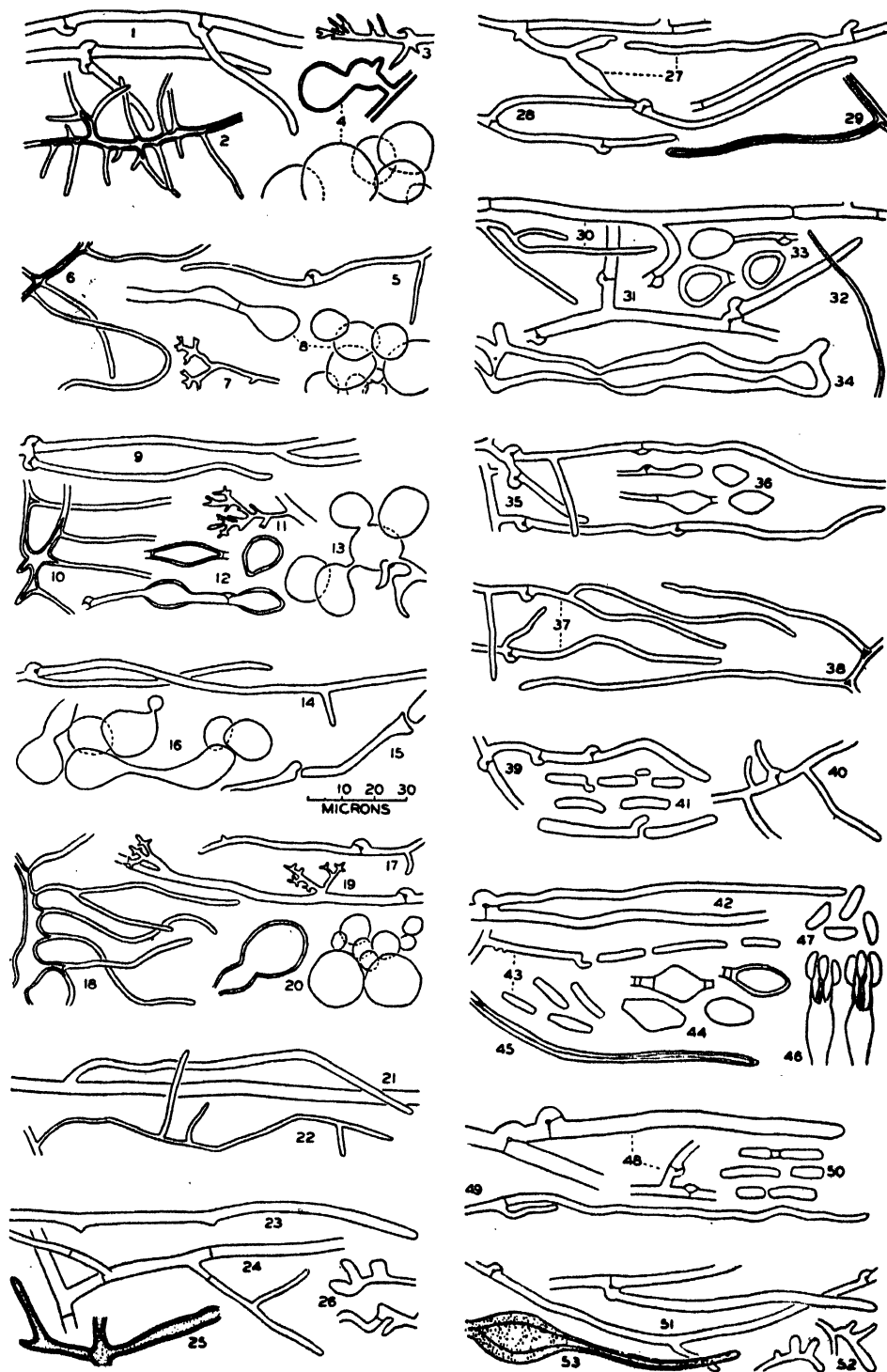
FIGS. 37 TO 38. *Lenzites betulina*. FIG. 37. *Hyphae from advancing zone*. FIG. 38. *Fiber hypha*.

FIGS. 39 TO 41. *Lenzites saepiaria*. FIG. 39. *Hypha from advancing zone*. FIG. 40. *Aerial hypha*. FIG. 41. *Oidia*.

FIGS. 42 TO 47. *Lenzites trabea*. FIG. 42. *Hypha from advancing zone*. FIG. 43. *Oidia*. FIG. 44. *Chlamydospores*. FIG. 45. *Fiber hypha*. FIG. 46. *Basidia*. FIG. 47. *Basidiospores*.

FIGS. 48 TO 50. *Merulius lacrymans*. FIG. 48. *Hyphae from aerial mycelium*. FIG. 49. *Hypha from 'oidial strain'*. FIG. 50. *Oidia*.

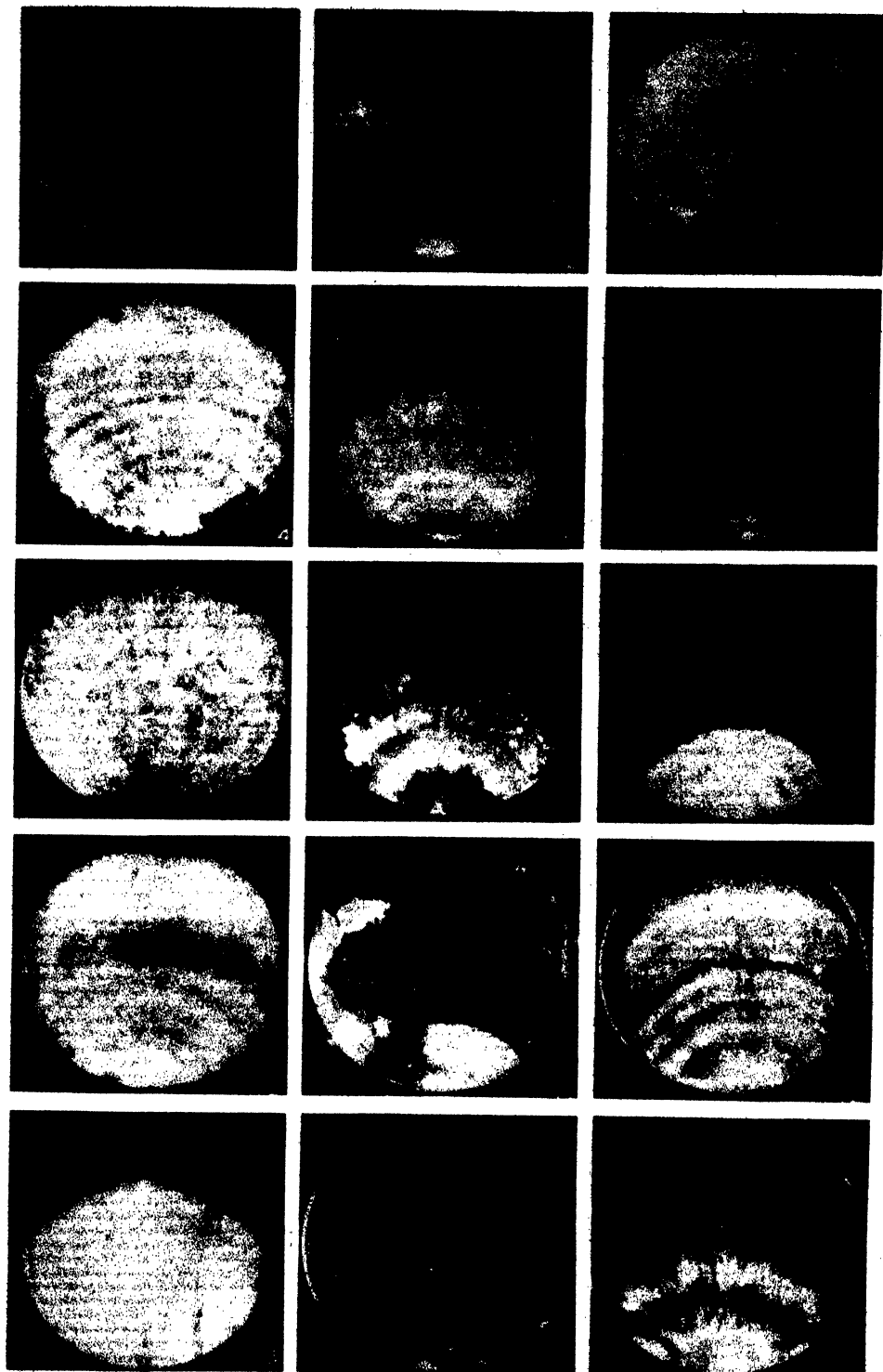
FIGS. 51 TO 53. *Omphalia campanella*. FIG. 51. *Hyphae from advancing zone*. FIG. 52. *Hyphae from pseudoparenchymatous layer*. FIG. 53. *Brown hyphae showing swelling*.



## PLATE VII

FIGS. 1 to 15. *Cultures grown on malt agar in the dark.*

- FIG. 1. *Omphalia campanella*, 11977, six weeks old.
- FIG. 2. *Peniophora gigantea*, 10249, two weeks old.
- FIG. 3. *Pholiota adiposa*, 8457, four weeks old.
- FIG. 4. *Pleurotus ostreatus*, F2949, four weeks old.
- FIG. 5. *Pleurotus ulmarius*, F326, four weeks old.
- FIG. 6. *Polyporus abietinus*, F7379, six weeks old.
- FIG. 7. *Polyporus adustus*, 11810, two weeks old.
- FIG. 8. *Polyporus albellus*, F7532, two weeks old.
- FIG. 9. *Polyporus amorphus*, 8552, six weeks old.
- FIG. 10. *Polyporus anceps*, 8264, two weeks old.
- FIG. 11. *Polyporus arcularius*, 10447, four weeks old.
- FIG. 12. *Polyporus balsameus*, F936, six weeks old.
- FIG. 13. *Polyporus Berkeleyi*, F2952, four weeks old.
- FIG. 14. *Polyporus betulinus*, 9339, six weeks old.
- FIG. 15. *Polyporus borealis*, 11685, four weeks old.



## PLATE VIII

FIGS. 1 TO 50. All  $\times 455$ .

FIGS. 1 TO 2. *Peniophora gigantea*. FIG. 1. *Hyphae from advancing zone*. FIG. 2. *Oidia*.  
FIGS. 3 TO 6. *Pholiota adiposa*. FIG. 3. *Hyphae from advancing zone*. FIG. 4. *Aerial hypha*.  
FIG. 5. *Conidiophores*. FIG. 6. *Conidia*.

FIGS. 7 TO 9. *Pleurotus ostreatus*. FIG. 7. *Hypha from advancing zone*. FIG. 8. *Aerial hyphae and hyphal segments*. FIG. 9. *Fiber hypha*.

FIG. 10. *Pleurotus ulmarius*. FIG. 10. *Hyphae from advancing zone*.

FIGS. 11 TO 14. *Polyporus abietinus*. FIG. 11. *Hyphae from advancing zone*. FIG. 12. *Aerial hypha*. FIG. 13. *Fiber hypha*. FIG. 14. *Capitate incrusted cystidia*.

FIGS. 15 TO 18. *Polyporus adustus*. FIG. 15. *Hypha from advancing zone*. FIG. 16. *Oidia from nodose-septate hypha*. FIG. 17. *Oidiophore with simple septa*. FIG. 18. *Oidia from hypha with simple septa*.

FIGS. 19 TO 21. *Polyporus albellus*. FIG. 19. *Hypha from advancing zone*. FIG. 20. *Contorted incrusted hyphal tips*. FIG. 21. *Chlamydospores*.

FIGS. 22 TO 24. *Polyporus amorphus*. FIG. 22. *Hypha from advancing zone*. FIG. 23. *Fiber hypha*. FIG. 24. *Swellings on submerged hyphae*.

FIGS. 25 TO 28. *Polyporus anceps*. FIG. 25. *Hypha from advancing zone*. FIG. 26. *Fiber hyphae*. FIG. 27. *Basidia*. FIG. 28. *Basidiospores*.

FIGS. 29 TO 32. *Polyporus arcularius*. FIG. 29. *Hypha from advancing zone*. FIG. 30. *Fiber hypha*. FIG. 31. *Hyphae from skinlike layer*. FIG. 32. *Chlamydospores*.

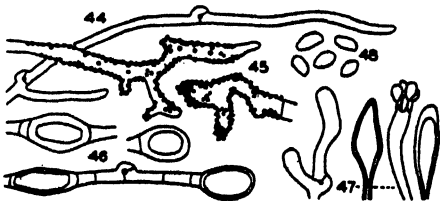
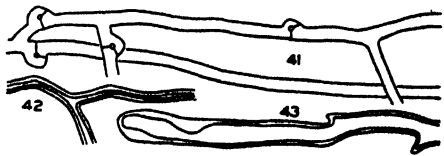
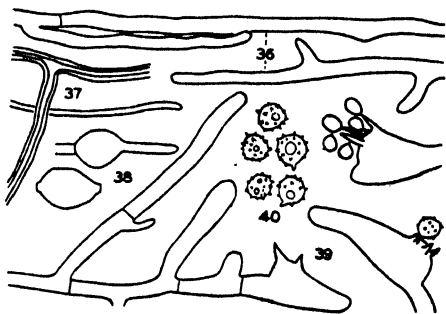
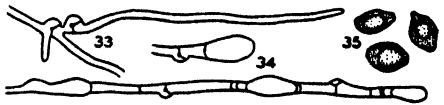
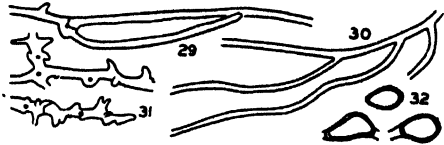
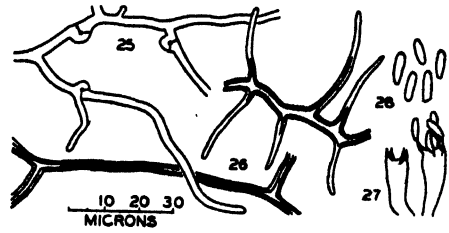
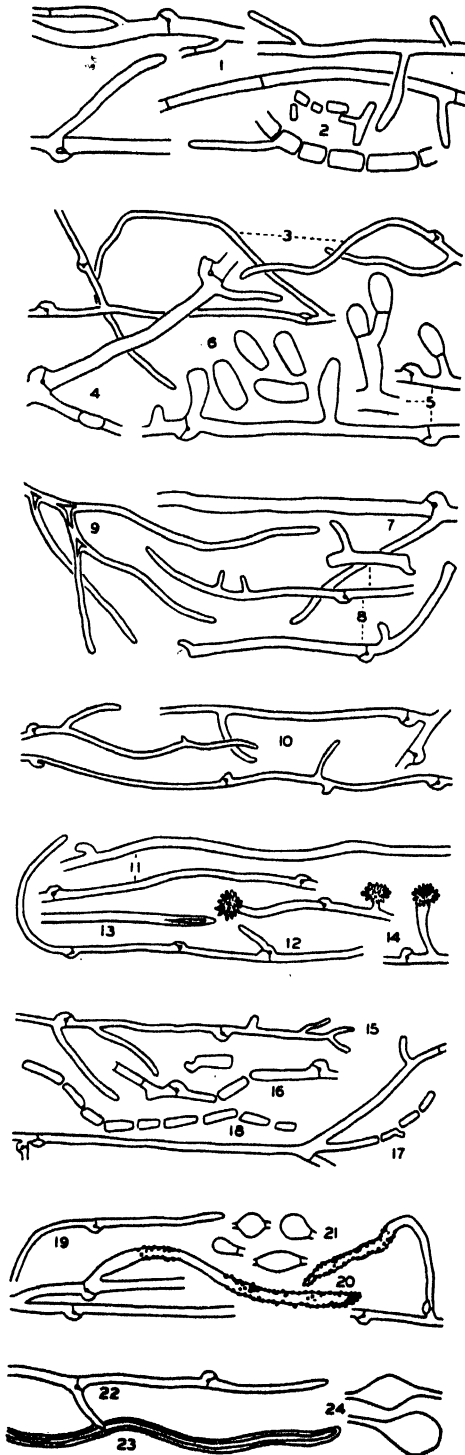
FIGS. 33 TO 35. *Polyporus balsameus*. FIG. 33. *Hypha from advancing zone*. FIG. 34. *Hyphae bearing chlamydospores*. FIG. 35. *Mature brown chlamydospores*.

FIGS. 36 TO 39. *Polyporus Berkeleyi*. FIG. 36. *Hyphae from advancing zone*. FIG. 37. *Fiber hypha*. FIG. 38. *Chlamydospores*. FIG. 39. *Conidiophores*. FIG. 40. *Conidia*.

FIGS. 41 TO 43. *Polyporus betulinus*. FIG. 41. *Hypha from advancing zone*. FIG. 42. *Fiber hypha*. FIG. 43. *Aerial hypha*.

FIGS. 44 TO 48. *Polyporus borealis*. FIG. 44. *Hypha from advancing zone*. FIG. 45. *Contorted incrusted hyphal tips*. FIG. 46. *Chlamydospores*. FIG. 47. *Basidia and cystidia*. FIG. 48. *Basidiospores*.

FIGS. 49 TO 50. *Polyporus brumalis*. FIG. 49. *Hypha from advancing zone*. FIG. 50. *Fiber hypha*.

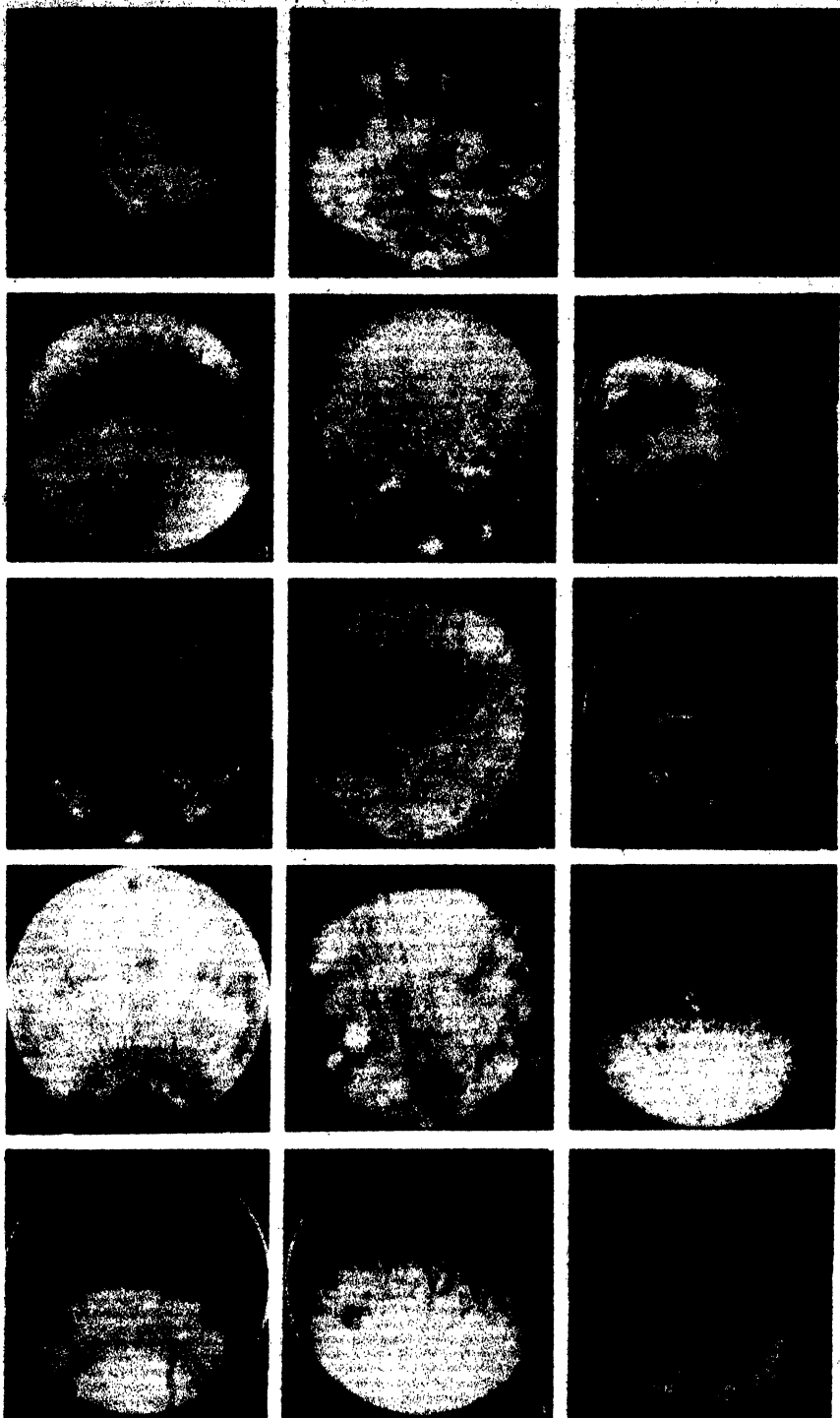




## PLATE IX

FIGS. 1 TO 15. *Cultures grown on malt agar in the dark.*

- FIG. 1. *Polyporus brumalis*, F1608, five weeks old.
- FIG. 2. *Polyporus cinnabarinus*, F1609, four weeks old.
- FIG. 3. *Polyporus circinatus*, 9416, six weeks old.
- FIG. 4. *Polyporus compactus*, F637, two weeks old.
- FIG. 5. *Polyporus conchifer*, 9343, four weeks old.
- FIG. 6. *Polyporus cuticularis*, F1969, six weeks old.
- FIG. 7. *Polyporus dichrous*, 8287, four weeks old.
- FIG. 8. *Polyporus distortus*, F7992, four weeks old.
- FIG. 9. *Polyporus dryadeus*, 8101, six weeks old.
- FIG. 10. *Polyporus dryophilus*, F2035, four weeks old.
- FIG. 11. *Polyporus dryophilus* var. *vulpinus*, F4394, four weeks old.
- FIG. 12. *Polyporus fibrillosus*, 8238, four weeks old.
- FIG. 13. *Polyporus fragilis*, 10227, six weeks old.
- FIG. 14. *Polyporus frondosus*, 10235, two weeks old.
- FIG. 15. *Polyporus fumosus*, 10257, four weeks old.



## PLATE X

FIGS. 1 TO 54. All  $\times 455$  except Figs. 36 and 51,  $\times 800$ .

FIGS. 1 TO 5. *Polyporus cinnabarinus*. FIG. 1. *Hypha from advancing zone*. FIG. 2. *Fiber hypha*. FIG. 3. *Chlamydospores*. FIG. 4. *Basidia*. FIG. 5. *Basidiospores*.

FIGS. 6 TO 10. *Polyporus circinatus*. FIG. 6. *Hyphae from advancing zone*. FIG. 7. *Dark brown aerial hyphae*. FIG. 8. *Swellings on aerial hyphae*. FIG. 9. *Hypha bearing chlamydosporelike structures*. FIG. 10. *Setae*.

FIGS. 11 TO 15. *Polyporus compactus*. FIG. 11. *Hyphae from advancing zone*. FIG. 12. *Fiber hyphae*. FIG. 13. *Chlamydospores*. FIG. 14. *Basidia*. FIG. 15. *Basidiospores*.

FIGS. 16 TO 17. *Polyporus conchifer*. FIG. 16. *Hyphae from advancing zone*. FIG. 17. *Fiber hypha*.

FIGS. 18 TO 19. *Polyporus cuticularis*. FIG. 18. *Hyphae from advancing zone*. FIG. 19. *Aerial hypha*.

FIG. 20. *Polyporus dichrous*. FIG. 20. *Hyphae from advancing zone*.

FIGS. 21 TO 25. *Polyporus distortus*. FIG. 21. *Hyphae from advancing zone*. FIG. 22. *Aerial hypha with chlamydospores*. FIG. 23. *Chlamydospores*. FIG. 24. *Clavate bodies*. FIG. 25. *Basidiospores*,  $\times 800$ .

FIGS. 26 TO 27. *Polyporus dryadeus*. FIG. 26. *Hyphae from advancing zone*. FIG. 27. *Hyphae from aerial mycelium*.

FIGS. 28 TO 30. *Polyporus dryophilus*. FIG. 28. *Hypha from advancing zone*. FIG. 29. *Pale aerial hypha*. FIG. 30. *Brown aerial hypha*.

FIGS. 31 TO 35. *Polyporus dryophilus var. vulpinus*. FIG. 31. *Hypha from advancing zone*. FIG. 32. *Aerial hypha*. FIG. 33. *Hyphae from skinlike layer*. FIG. 34. *Basidia*. FIG. 35. *Basidiospores*.

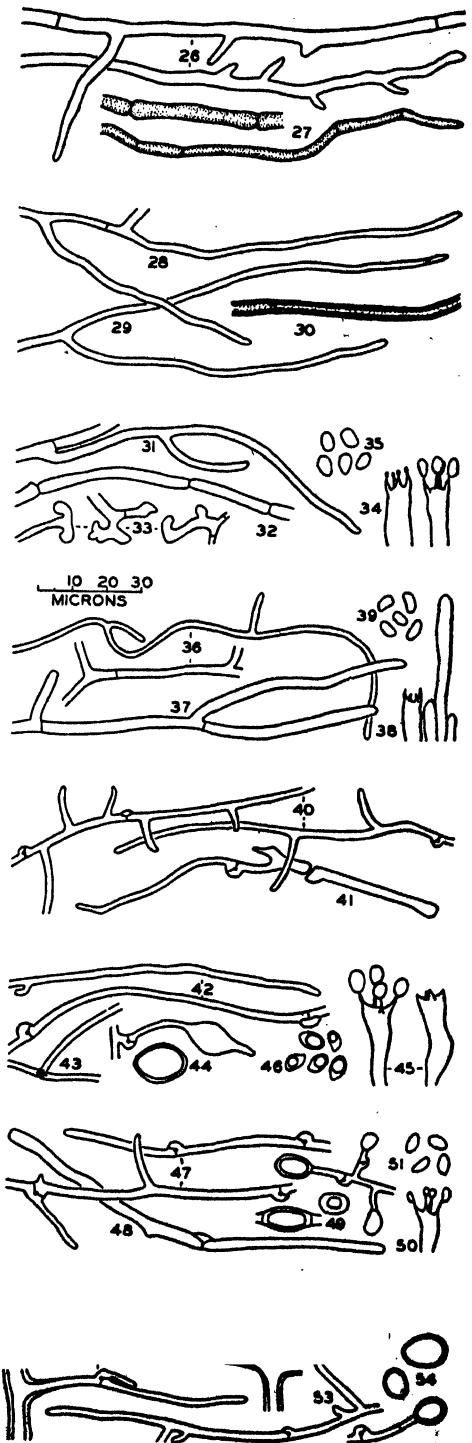
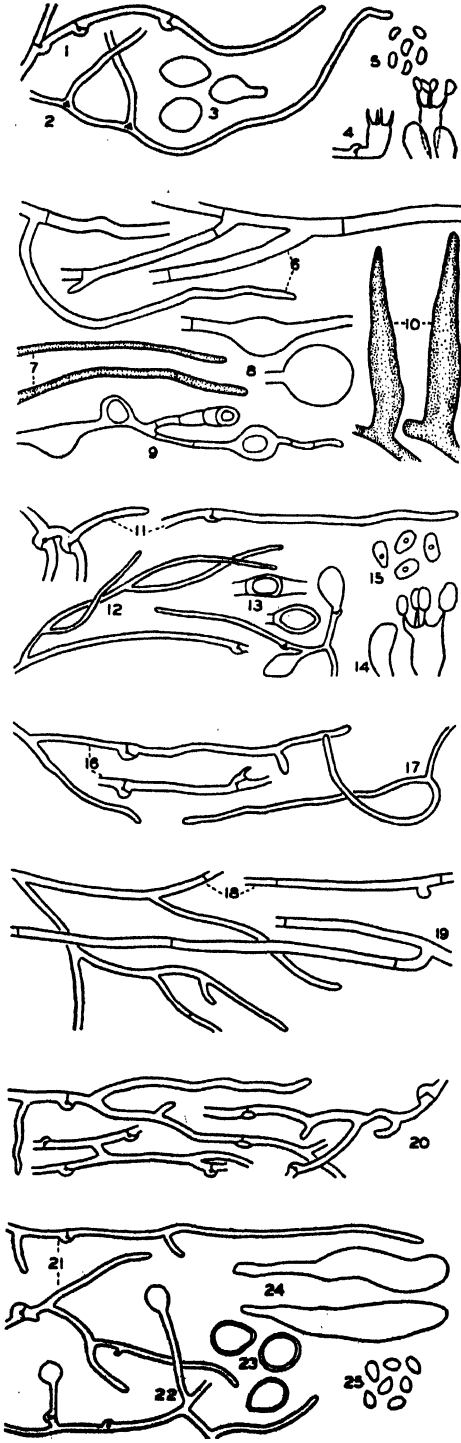
FIGS. 36 TO 39. *Polyporus fibrillosus*. FIG. 36. *Hyphae from advancing zone*. FIG. 37. *Aerial hypha*. FIG. 38. *Basidia and cystidium*. FIG. 39. *Basidiospores*.

FIGS. 40 TO 41. *Polyporus fragilis*. FIG. 40. *Hyphae from advancing zone*. FIG. 41. *Hyphal segment from aerial mycelium*.

FIGS. 42 TO 46. *Polyporus frondosus*. FIG. 42. *Hyphae from advancing zone*. FIG. 43. *Fiber hypha*. FIG. 44. *Chlamydospores*. FIG. 45. *Basidia*. FIG. 46. *Basidiospores*.

FIGS. 47 TO 51. *Polyporus fumosus*. FIG. 47. *Hyphae from advancing zone*. FIG. 48. *Hyphal segment from aerial mycelium*. FIG. 49. *Chlamydospores*. FIG. 50. *Basidium*. FIG. 51. *Basidiospores*,  $\times 800$ .

FIGS. 52 TO 54. *Polyporus galactinus*. FIG. 52. *Hyphae from advancing zone*. FIG. 53. *Aerial hypha*. FIG. 54. *Chlamydospores*.



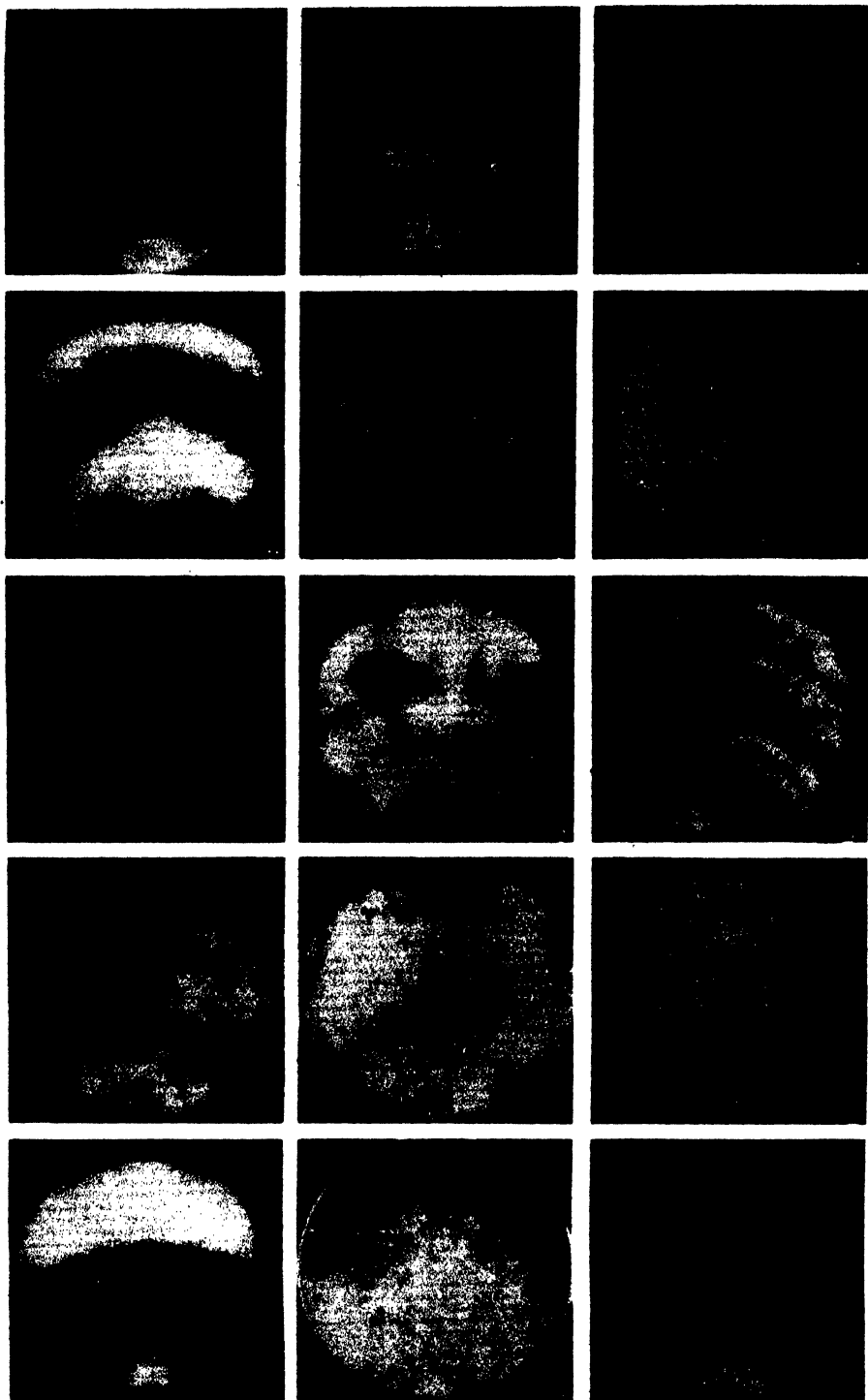
## PLATE XI

FIGS. 1 TO 15. *Cultures grown on malt agar in the dark.*

- FIG. 1. *Polyporus galactinus*, F3494, two weeks old.
- FIG. 2. *Polyporus gilvus*, F1704, five weeks old.
- FIG. 3. *Polyporus glomeratus*, F3491, six weeks old.
- FIG. 4. *Polyporus graveolens*, F2954, four weeks old.
- FIG. 5. *Polyporus guttulatus*, F638, four weeks old.
- FIG. 6. *Polyporus hirsutus*, F1319, two weeks old.
- Fig. 7. *Polyporus mollis*, F6818, four weeks old.
- FIG. 8. *Polyporus montanus*, F8268, six weeks old.
- FIG. 9. *Polyporus obtusus*, F6798, six weeks old.
- FIG. 10. *Polyporus palustris*, F2964, four weeks old.
- FIG. 11. *Polyporus pargamenus*, F8016, four weeks old.
- FIG. 12. *Polyporus pubescens*, F3058, two weeks old.
- FIG. 13. *Polyporus radiatus*, 10207, four weeks old.
- FIG. 14. *Polyporus resinosus*, F2063, two weeks old.
- FIG. 15. *Polyporus rutilans*, 11607, four weeks old.









## PLATE XII

FIGS. 1 TO 54. All  $\times 455$ , except Figs. 16, 17, 21, 22, 35, 36,  $\times 800$ .

FIGS. 1 TO 6. *Polyporus gilvus*. FIG. 1. *Hyphae from advancing zone*. FIG. 2. *Nodose-septate hypha from aerial mycelium*. FIG. 3. *Pale aerial hypha*. FIG. 4. *Dark brown aerial hypha*. FIG. 5. *Setae from fruit body*. FIG. 6. *Basidiospores*.

FIGS. 7 TO 10. *Polyporus glomeratus*. FIG. 7. *Hypha from advancing zone*. FIG. 8. *Aerial hyphae*. FIG. 9. *Hyphae from skinlike layer*. FIG. 10. *Setal hypha*.

FIGS. 11 TO 14. *Polyporus graveolens*. FIG. 11. *Hypha from advancing zone*. FIG. 12. *Fiber hypha*. FIG. 13. *Cuticular cells*. FIG. 14. *Oidia*.

FIGS. 15 TO 17. *Polyporus guttulatus*. FIG. 15. *Hyphae from advancing zone*. FIG. 16. *Conidiophores*,  $\times 800$ . FIG. 17. *Conidia*,  $\times 800$ .

FIGS. 18 TO 22. *Polyporus hirsutus*. FIG. 18. *Hyphae from advancing zone*. FIG. 19. *Fiber hypha*. FIG. 20. *Chlamydospores*. FIG. 21. *Basidium*,  $\times 800$ . FIG. 22. *Basidiospores*,  $\times 800$ .

FIGS. 23 TO 24. *Polyporus mollis*. FIG. 23. *Hyphae from advancing zone*. FIG. 24. *Aerial hypha*.

FIGS. 25 TO 27. *Polyporus montanus*. FIG. 25. *Hyphae from advancing zone*. FIG. 26. *Fiber hyphae*. FIG. 27. *Chlamydospores*.

FIGS. 28 TO 31. *Polyporus obtusus*. FIG. 28. *Hypha from advancing zone*. FIG. 29. *Chlamydospores*. FIG. 30. *Aerial hypha*. FIG. 31. *Fiber hypha*.

FIGS. 32 TO 36. *Polyporus palustris*. FIG. 32. *Hypha from advancing zone*. FIG. 33. *Fiber hyphae*. FIG. 34. *Chlamydospores*. FIG. 35. *Basidia*,  $\times 800$ . FIG. 36. *Basidiospores*,  $\times 800$ .

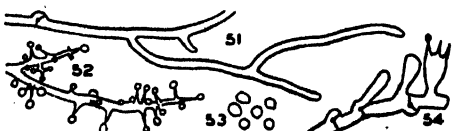
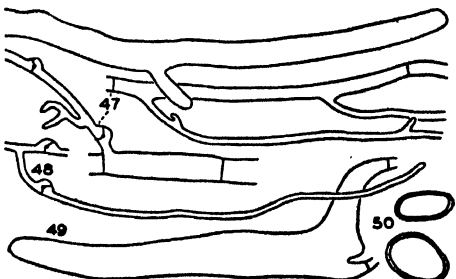
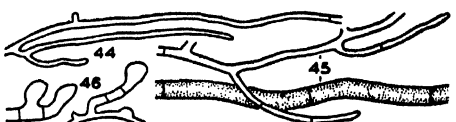
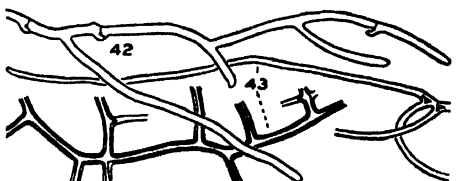
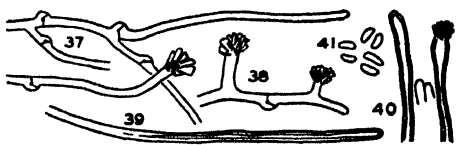
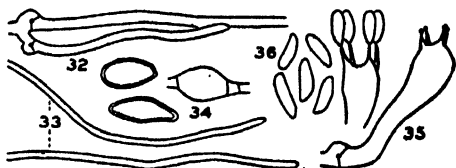
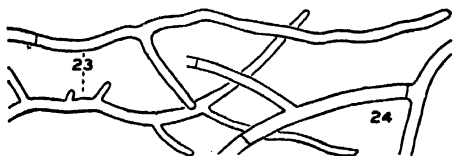
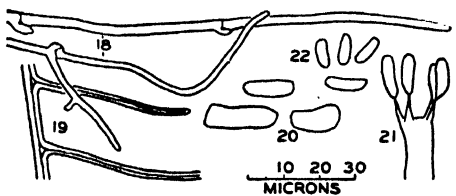
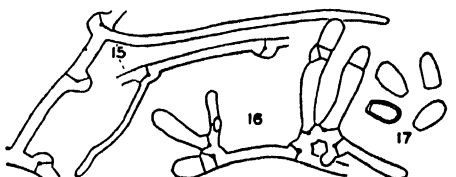
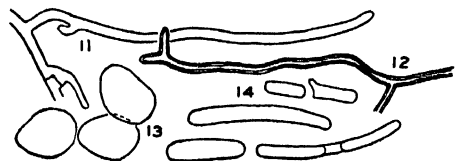
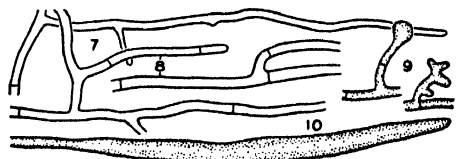
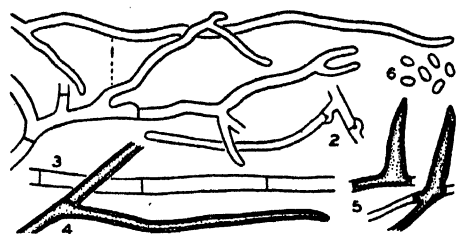
FIGS. 37 TO 41. *Polyporus pargamenus*. FIG. 37. *Hypha from advancing zone*. FIG. 38. *Capitate incrustated cystidia*. FIG. 39. *Fiber hypha*. FIG. 40. *Cystidia from fruit body*. FIG. 41. *Basidiospores*.

FIGS. 42 TO 43. *Polyporus pubescens*. FIG. 42. *Hypha from advancing zone*. FIG. 43. *Fiber hyphae*.

FIGS. 44 TO 46. *Polyporus radiatus*. FIG. 44. *Hypha from advancing zone*. FIG. 45. *Aerial hyphae*. FIG. 46. *Hyphae from skinlike layer*.

FIGS. 47 TO 50. *Polyporus resinosus*. FIG. 47. *Hyphae from advancing zone*. FIG. 48. *Aerial hypha*. FIG. 49. *Lactiferous cell*. FIG. 50. *Chlamydospores*.

FIGS. 51 TO 54. *Polyporus rutilans*. FIG. 51. *Hypha from advancing zone*. FIG. 52. *Conidiophores*. FIG. 53. *Conidia*. FIG. 54. *Basidia*.



## PLATE XIII

FIGS. 1 TO 12. *Cultures grown on malt agar in the dark.*

FIG. 1. *Polyporus Schweinitzii*, 9420, four weeks old.

FIG. 2. *Polyporus semipileatus*, F7335, six weeks old.

FIG. 3. *Polyporus squamosus*, 10789, six weeks old.

FIG. 4. *Polyporus subcartilagineus*, 9417, four weeks old.

FIG. 5. *Polyporus sulphureus*, F3474, two weeks old.

FIG. 6. *Polyporus tuberaster*, F6623, six weeks old.

FIG. 7. *Polyporus Tulipiferae*, F8049, two weeks old.

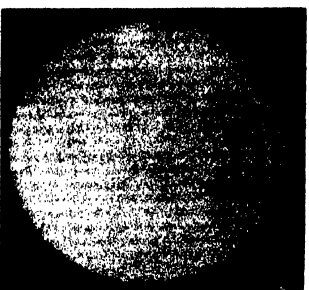
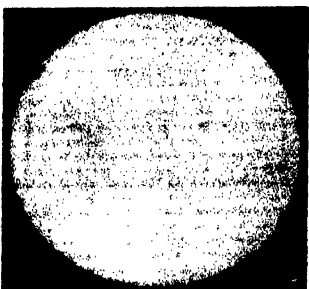
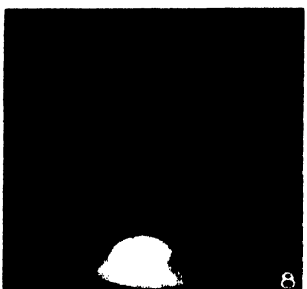
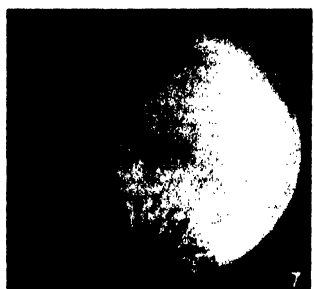
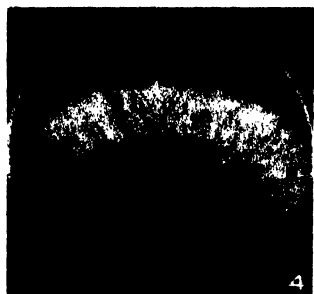
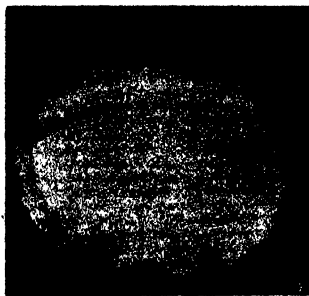
FIG. 8. *Polyporus umbellatus*, F7336, six weeks old.

FIG. 9. *Polyporus versicolor*, 8183, three weeks old.

FIG. 10. *Polyporus volvatus*, F6814, four weeks old.

FIG. 11. *Polyporus zonatus*, F7338, one week old.

FIG. 12. *Polyporus zonatus*, F7390, six weeks old.



## PLATE XIV

FIGS. 1 TO 31. All  $\times 455$ .

FIGS. 1 TO 5. *Polyporus Schweinitzii*. FIG. 1. *Hyphae from advancing zone*. FIG. 2. *Hyaline aerial mycelium*. FIG. 3. *Yellow aerial hypha*. FIG. 4. *Chlamydospores*. FIG. 5. *Brown aerial hypha*.

FIGS. 6 TO 7. *Polyporus semipileatus*. FIG. 6. *Hyphae from advancing zone*. FIG. 7. *Contorted incrusting hyphal tips*.

FIGS. 8 TO 10. *Polyporus squamosus*. FIG. 8. *Hypha from advancing zone*. FIG. 9. *Oidia*. FIG. 10. *Hyphae from skinlike area*.

FIG. 11. *Polyporus subcartilagineus*. FIG. 11. *Hyphae from advancing zone*.

FIGS. 12 TO 15. *Polyporus sulphureus*. FIG. 12. *Hyphae from advancing zone*. FIG. 13. *Conidiophore*. FIG. 14. *Conidia*. FIG. 15. *Chlamydospores*.

FIGS. 16 TO 18. *Polyporus tuberaster*. FIG. 16. *Hypha from advancing zone*. FIG. 17. *Fiber hypha*. FIG. 18. *Hyphae from pseudoparenchymatous layer*.

FIGS. 19 TO 20. *Polyporus Tulipiferae*. FIG. 19. *Hyphae from advancing zone*. FIG. 20. *Nonstaining hyphae*.

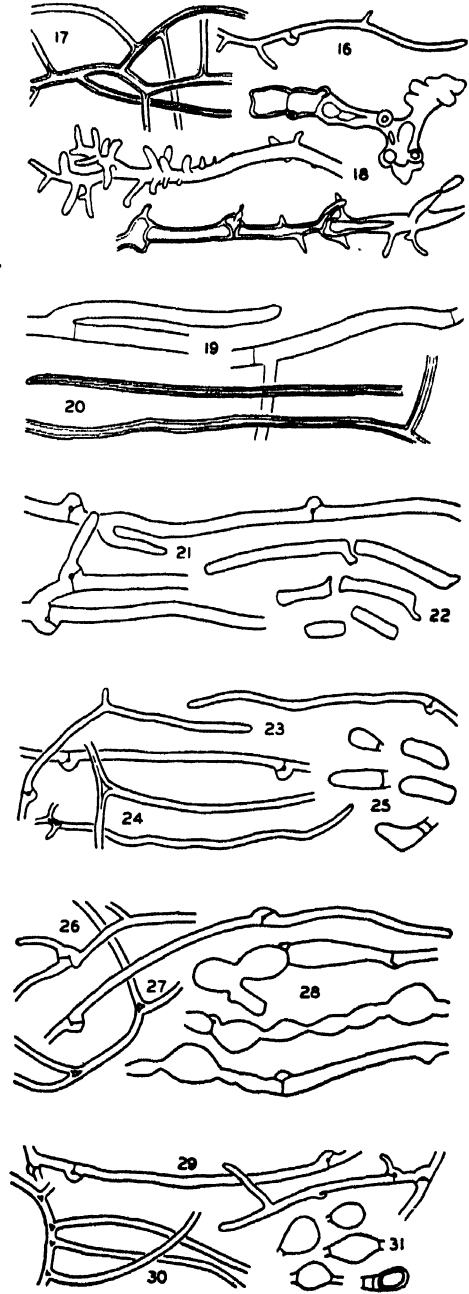
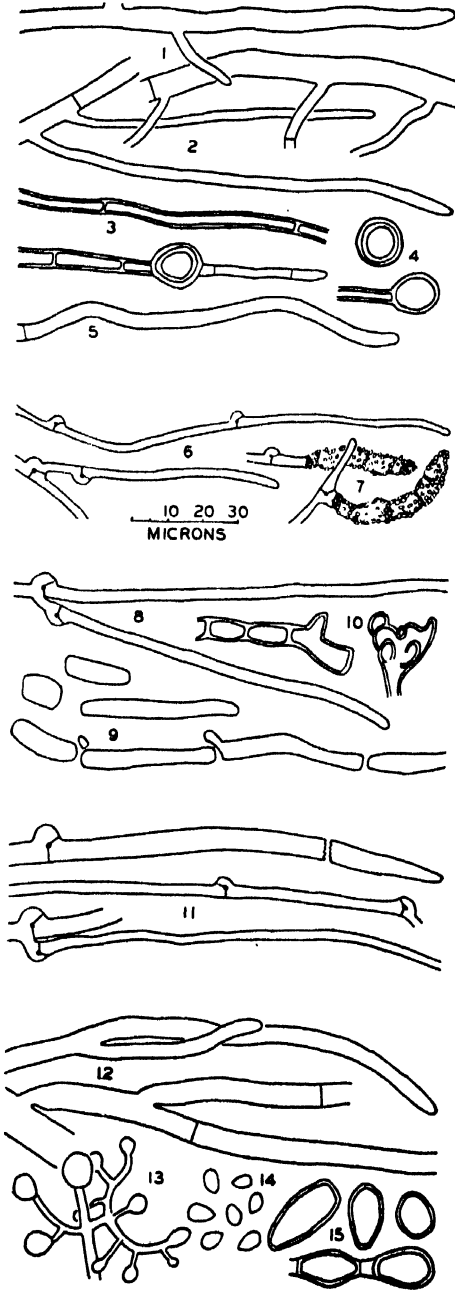
FIGS. 21 TO 22. *Polyporus umbellatus*. FIG. 21. *Hyphae from advancing zone*. FIG. 22. *Oidia*.

FIGS. 23 TO 25. *Polyporus versicolor*. FIG. 23. *Hyphae from advancing zone*. FIG. 24. *Fiber hyphae*. FIG. 25. *Chlamydospores*.

FIGS. 26 TO 28. *Polyporus volvatus*. FIG. 26. *Hyphae from advancing zone*. FIG. 27. *Fiber hypha*. FIG. 28. *Hyphae with swellings*.

FIGS. 29 TO 31. *Polyporus zonatus*. FIG. 29. *Hyphae from advancing zone*. FIG. 30. *Fiber hypha*. FIG. 31. *Chlamydospores*.

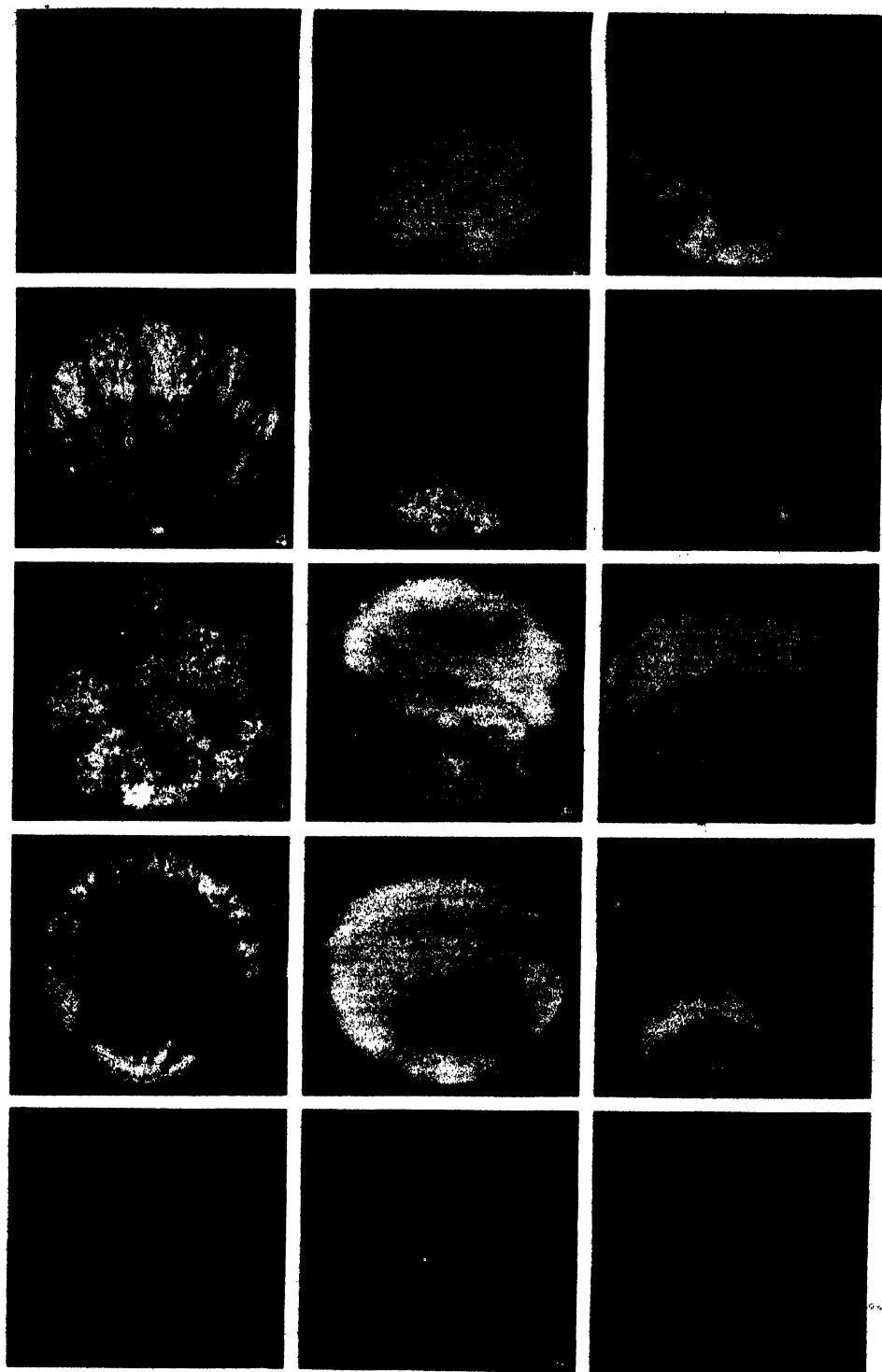
PLATE XIV



## PLATE XV

FIGS. 1 TO 15. *Cultures grown on malt agar in the dark.*

- FIG. 1. *Poria albidipellucida*, 11692, *two weeks old.*
- FIG. 2. *Poria asiatica*, 17118, *six weeks old.*
- FIG. 3. *Poria carbonica*, 8269, *five weeks old.*
- FIG. 4. *Poria ferrea*, 9254, *five weeks old.*
- FIG. 5. *Poria ferrugineo-fusca*, F7340, *four weeks old.*
- FIG. 6. *Poria ferruginosa*, 11253, *six weeks old.*
- FIG. 7. *Poria monticola*, 9251, *six weeks old.*
- FIG. 8. *Poria obliqua*, F7449, *six weeks old.*
- FIG. 9. *Poria punctata*, F1402, *four weeks old.*
- FIG. 10. *Poria rufa*, 16615, *six weeks old.*
- FIG. 11. *Poria subacida*, F598, *two weeks old.*
- FIG. 12. *Poria tsugina*, 9243, *six weeks old.*
- FIG. 13. *Poria Vaillantii*, 11740, *six weeks old.*
- FIG. 14. *Poria Weirii*, 9323, *two weeks old.*
- FIG. 15. *Poria xantha*, 16088, *four weeks old.*





## PLATE XVI

FIGS. 1 TO 48. All  $\times 455$ , except Figs. 23 and 24,  $\times 800$ .

FIGS. 1 TO 5. *Poria albipellucida*. FIG. 1. *Hypha from advancing zone*. FIG. 2. *Thick-walled aerial hyphae*. FIG. 3. *Nodose-septate aerial hyphae*. FIG. 4. *Chlamydospores*. FIG. 5. *Oidia*.

FIGS. 6 TO 7. *Poria asiatica*. FIG. 6. *Hyphae from advancing zone*. FIG. 7. *Chlamydospores*.

FIGS. 8 TO 11. *Poria carbonica*. FIG. 8. *Hyphae from advancing zone*. FIG. 9. *Hypha from aerial mycelium*. FIG. 10. *Conidiophores and conidia*. FIG. 11. *Chlamydospores*.

FIGS. 12 TO 13. *Poria ferrea*. FIG. 12. *Hypha from advancing zone*. FIG. 13. *Hyphae from aerial mycelium*.

FIGS. 14 TO 15. *Poria ferrugineo-fusca*. FIG. 14. *Hyphae from advancing zone*. FIG. 15. *Setal hyphae*.

FIGS. 16 TO 18. *Poria ferruginosa*. FIG. 16. *Hypha from advancing zone*. FIG. 17. *Aerial hyphae showing anastomosis*. FIG. 18. *Setae*.

FIGS. 19 TO 24. *Poria monticola*. FIG. 19. *Hyphae from advancing zone*. FIG. 20. *Thick-walled hypha from aerial mycelium*. FIG. 21. *Chlamydospores*. FIG. 22. *Hypha from submerged mycelium*. FIG. 23. *Basidia*,  $\times 800$ . FIG. 24. *Basidiospores*,  $\times 800$ .

FIGS. 25 TO 29. *Poria obliqua*. FIG. 25. *Hypha from advancing zone*. FIG. 26. *Aerial hyphae*. FIG. 27. *Setae from aerial mycelium*. FIG. 28. *Basidia and setae from fruiting surface*. FIG. 29. *Basidiospores*.

FIGS. 30 TO 32. *Poria punctata*. FIG. 30. *Hyphae from advancing zone*. FIG. 31. *Brown aerial hyphae*. FIG. 32. *Hypha from submerged mycelium*.

FIGS. 33 TO 34. *Poria rufa*. FIG. 33. *Hyphae from advancing zone*. FIG. 34. *Hypha from aerial mycelium*.

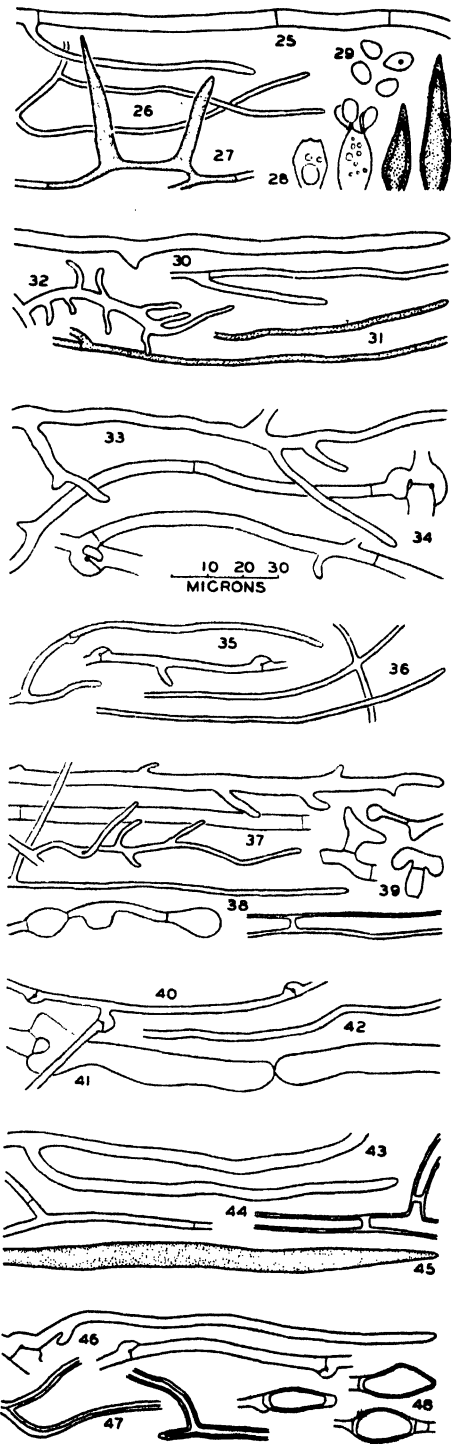
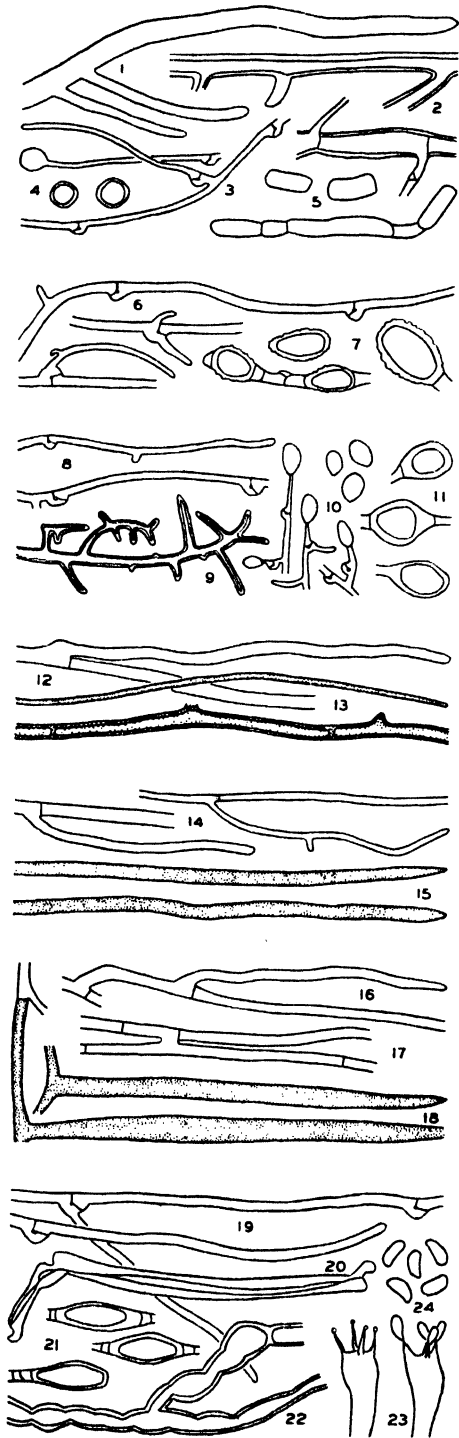
FIGS. 35 TO 36. *Poria subacida*. FIG. 35. *Hyphae from advancing zone*. FIG. 36. *Fiber hyphae*.

FIGS. 37 TO 39. *Poria tsugina*. FIG. 37. *Hyphae from advancing zone*. FIG. 38. *Aerial hyphae*. FIG. 39. *Hyphae from crustose area*.

FIGS. 40 TO 42. *Poria Vaillantii*. FIG. 40. *Hypha from advancing zone*. FIG. 41. *Broad hypha from submerged mycelium*. FIG. 42. *Fiber hypha from fruit body*.

FIGS. 43 TO 45. *Poria Weirii*. FIG. 43. *Hypha from advancing zone*. FIG. 44. *Aerial hyphae*. FIG. 45. *End of setal hypha*.

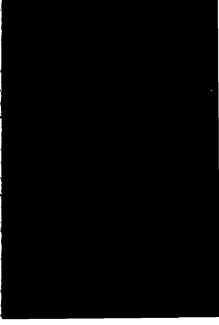
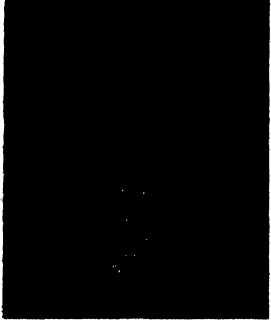
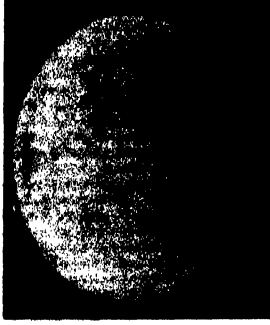
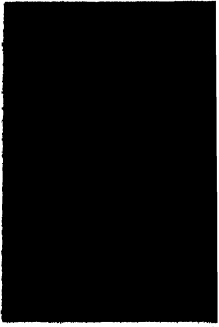
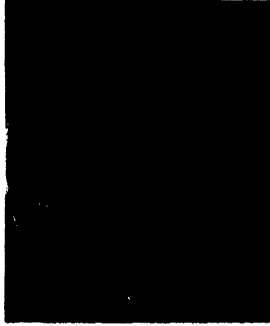
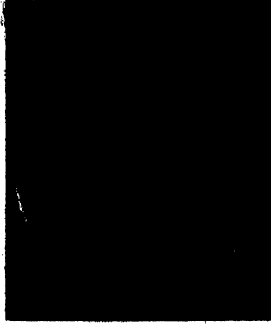
FIGS. 46 TO 48. *Poria xantha*. FIG. 46. *Hyphae from advancing zone*. FIG. 47. *Thick-walled hyphae*. FIG. 48. *Chlamydospores*.



## PLATE XVII

FIGS. 1 TO 12. *Cultures grown on malt agar in the dark.*

- FIG. 1. *Schizophyllum commune*, F2961, *four weeks old.*
- FIG. 2. *Stereum abietinum*, 16091, *six weeks old.*
- FIG. 3. *Stereum Murraili*, F1362, *six weeks old.*
- FIG. 4. *Stereum sanguinolentum*, 9330, *four weeks old.*
- FIG. 5. *Trametes americana*, 8189, *six weeks old.*
- FIG. 6. *Trametes americana*, 11789, *six weeks old.*
- FIG. 7. *Trametes heteromorpha*, 11991, *six weeks old.*
- FIG. 8. *Trametes sepium*, 11987, *four weeks old.*
- FIG. 9. *Trametes serialis*, 10916, *four weeks old.*
- FIG. 10. *Trametes suaveolens*, F3523, *four weeks old.*
- FIG. 11. *Trametes tenuis*, 9265, *six weeks old.*
- FIG. 12. *Trametes variiformis*, 16098, *six weeks old.*

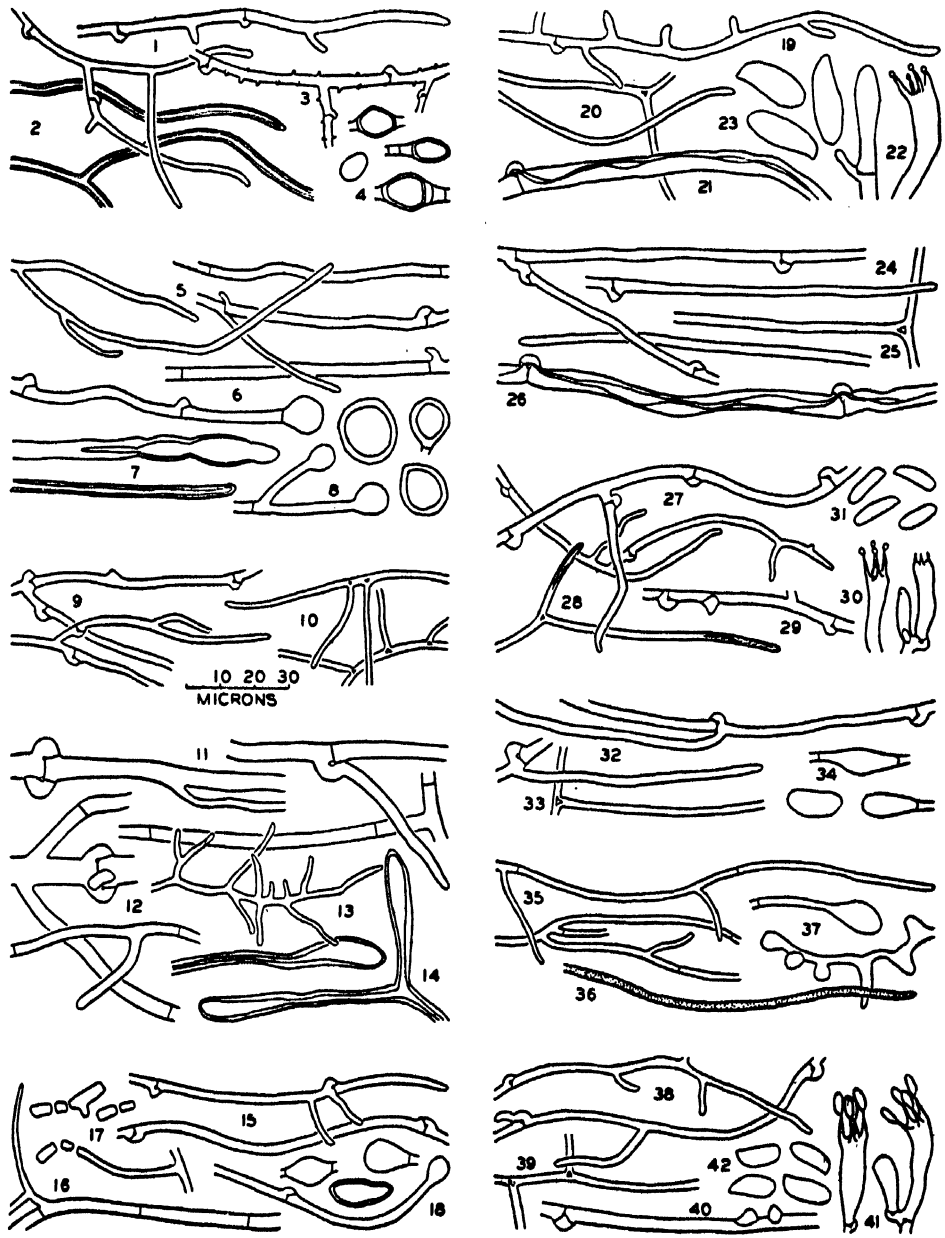


## PLATE XVIII

FIGS. 1 TO 41. All  $\times 455$ , except Figs. 23, 31, 42,  $\times 800$ .

- FIGS. 1 TO 4. *Schizophyllum commune*. FIG. 1. *Hypha from advancing zone*. FIG. 2. *Aerial hyphae with thickened walls*. FIG. 3. *Aerial hypha showing projections*. FIG. 4. *Chlamydospores*.
- FIGS. 5 TO 8. *Stereum abietinum*. FIG. 5. *Hyphae from advancing zone*. FIG. 6. *Aerial hyphae showing types of septation*. FIG. 7. *Thick-walled aerial hyphae*. FIG. 8. *Chlamydospores*.
- FIGS. 9 TO 10. *Stereum Murraii*. FIG. 9. *Hyphae from advancing zone*. FIG. 10. *Fiber hyphae*.
- FIGS. 11 TO 14. *Stereum sanguinolentum*. FIG. 11. *Hyphae from advancing zone*. FIG. 12. *Aerial hyphae*. FIG. 13. *Much-branched aerial hypha*. FIG. 14. *Brown conducting cells*.
- FIGS. 15 TO 18. *Trametes americana*. FIG. 15. *Hyphae from diploid colony*. FIG. 16. *Hypha from haploid colony*. FIG. 17. *Oidia*. FIG. 18. *Chlamydospores*.
- FIGS. 19 TO 23. *Trametes heteromorpha*. FIG. 19. *Hypha from advancing zone*. FIG. 20. *Fiber hyphae*. FIG. 21. *Thick-walled hypha from aerial mycelium*. FIG. 22. *Basidia*. FIG. 23. *Basidiospores*,  $\times 800$ .
- FIGS. 24 TO 26. *Trametes sepium*. FIG. 24. *Hyphae from advancing zone*. FIG. 25. *Fiber hyphae*. FIG. 26. *Thick-walled aerial hypha*.
- FIGS. 27 TO 31. *Trametes serialis*. FIG. 27. *Hyphae from advancing zone*. FIG. 28. *Fiber hypha*. FIG. 29. *Hypha from aerial mycelium*. FIG. 30. *Basidia*. FIG. 31. *Basidiospores*,  $\times 800$ .
- FIGS. 32 TO 34. *Trametes suaveolens*. FIG. 32. *Hyphae from advancing zone*. FIG. 33. *Fiber hypha*. FIG. 34. *Chlamydospores*.
- FIGS. 35 TO 37. *Trametes tenuis*. FIG. 35. *Hyphae from advancing zone*. FIG. 36. *Brown aerial hypha*. FIG. 37. *Swollen cells on aerial hyphae*.
- FIGS. 38 TO 42. *Trametes variiformis*. FIG. 38. *Hyphae from advancing zone*. FIG. 39. *Fiber hypha*. FIG. 40. *Hypha from aerial mycelium*. FIG. 41. *Basidia*. FIG. 42. *Basidiospores*,  $\times 800$ .

PLATE XVIII





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## ASCORBIC ACID IN POTATOES GROWN IN NEW BRUNSWICK<sup>1</sup>

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### Abstract

Values for the ascorbic acid content in the tubers of nine potato varieties have been compared. The effects of different fertilizers have been studied. Location of plots throughout the province was considered. Changes in ascorbic acid content due to tuber maturity and storage time have been measured at regular intervals. The relative amounts of ascorbic acid in the stem and eye ends of tubers have been found. Effects of top-killers on the ascorbic acid content of tubers were noted. No relationship was observed between the ascorbic acid content and either variety or fertilizer. The highest values for ascorbic acid were obtained in August. Losses during maturity and storage were continuous and fairly regular. Top-killers produced no apparent effect on the ascorbic acid content of tubers. The eye end of the potato tuber contained approximately 20% more ascorbic acid than the stem end.

### Introduction

A survey has been made of the ascorbic acid content of certain commercial varieties of potatoes grown in New Brunswick.

The potato is one of New Brunswick's major crops. Accordingly, its food value and vitamin content assume a special significance in this province. The potato does not contain a high concentration of ascorbic acid but because of its widespread distribution and use it is one of the most valuable dietary sources of this vitamin.

The literature contains many conflicting findings in regard to the amounts of ascorbic acid in tubers of different varieties, as well as in tubers grown and stored under different conditions.

Esselen, Lyons, and Fellers (2) reported that the amount of ascorbic acid varied with variety, as well as with the season, but they found that geographical location had little or no effect on ascorbic acid content. On the other hand, Karikka, Dudgeon, and Hauck (3) observed varietal differences and also marked variation in ascorbic acid content among potatoes grown in different localities. Murphy (9) found differences in the vitamin C content of stored potatoes of different varieties. Lyons and Fellers (7) found differences within a variety to be greater than between varieties.

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The effect of fertilizer has been studied extensively. Westas (19) found that fertilizer had no effect on the ascorbic acid concentration, as did also Scheunert, Reschke, and Kohlemann (15, 16) and Baker, Parkinson, and Lampitt (1). Nehring (11) claimed that the influence of single elements on vitamin C content had not been established. Later, Rangnekar (12) reported that up to a certain point vitamin C increased with an increase in manganese. Wachholder and Nehring (18) found that the ascorbic acid content was higher when potatoes were grown without fertilizer; but the Kentucky Agricultural Experiment Station Report No. 56 (4) showed that potatoes from plants receiving no fertilizer contained less ascorbic acid.

Nehring (11) and Wachholder and Nehring (18) suggested the importance of soil or local conditions other than fertilizer or variety in controlling the vitamin C concentration of potatoes. Lampitt, Baker, and Parkinson (5) found no significant difference in vitamin C concentration between potatoes of one variety grown on two types of soil.

Murphy (8) discussed the advantages and disadvantages of different storage temperatures. She recommended 50° F. for optimum retention of vitamin C. Karikka, Dudgeon, and Hauck (3) also favored 50° F. for storage.

The distribution of ascorbic acid in the tuber has also been the subject of several papers. Rolf (14) reported an uneven distribution and Murphy, Dove, and Akeley (10) spoke of the more concentrated bud end and the less concentrated stem end. Laurensen and Orth (6) found differences between the center and outer layers.

The effects of many other variables such as tuber maturity, size, shape, starch content, disease resistance, season, and locality have been discussed by many workers. Truscott, Johnstone, Drake, Van Haarlem, and Thomson (17) have considered several varieties in different years, both fresh and after storage. They found considerable variation between varieties and within varieties depending on the year of harvest.

Since no clear picture could be obtained from the literature it was felt that a survey of the ascorbic acid content of New Brunswick grown potatoes would be justified.

The work has been summarized in four parts, each part being considered separately. Generalizations follow, where possible.

Primarily, this survey was attempted in order to discover what differences, if any, could be observed in the ascorbic acid content of different varieties of New Brunswick potatoes under different conditions of growth and storage.

For the most part, the potatoes for this survey were grown at the Dominion Experimental Station at Fredericton. Those reported in Table III were grown in different localities throughout the province, but under the supervision of the Experimental Station.

The Green Mountain variety was chosen for all experiments except for the nine varieties reported in Table I.

All potatoes tested were from the 1946 crop. No weather records have been kept, although it is known that moisture and humidity have considerable effect on the size and nature of the tubers, so that indirectly the ascorbic acid may vary with the season. The potatoes were tested from July 1946 to August 1947.

### Method of Storing

The potatoes were harvested in October. After harvesting the tubers were stored in bins at the Experimental Station until January when they were transferred to a temperature of about 40° F., with a variation of  $\pm 2^\circ$  F.

### Method of Testing

The method of Roe (13) was used for determining the ascorbic acid in the potatoes. The procedure was that recommended for plant tissues. A weighed portion was ground in a Waring Blendor with 5% metaphosphoric acid containing 10% acetic acid. The extract was treated with acid-washed charcoal to oxidize the reduced ascorbic acid to dehydroascorbic acid. This extract and a standard solution of ascorbic acid were treated with dinitrophenylhydrazine, incubated for three hours at 37° C., and made up to volume with sulphuric acid. They were then compared colorimetrically, and the amount of ascorbic acid in the potato was calculated. This value included all the ascorbic acid present in either the reduced or oxidized form, but measured as dehydroascorbic acid.

Accordingly, for this paper, the term 'ascorbic acid' means the sum of 'converted' and 'free' dehydroascorbic acid.

A great many values for 'free' dehydroascorbic acid have also been obtained but these values have not been included in this paper.

Repeated recovery experiments and frequent testing of unknown standard solutions of ascorbic acid indicated that the tests were performed from day to day by the same workers during the whole period of investigation with an error not exceeding  $\pm 5\%$ .

### Method of Sampling

Samples for analysis were prepared by chopping whole potatoes (including peel) into small pieces, using a plastic knife. After thorough mixing, a sample weighing 5 to 10 gm. was tested.

### Method of Reporting

For each sample tested, percentage dry weights were obtained in duplicate by drying aliquots to constant weight in a drying oven at approximately 103° C. The values are reported in mgm. of ascorbic acid per 100 gm. of dry weight. The dry weight values have been used to express the changes in ascorbic acid independently of initial differences in moisture content or loss of water during storage. The percentage dry weights varied from 17 to 19% in July to 26 to 30% the following May.

### Preliminary Observations

Early in the season, potatoes from separate hills were analyzed. Groups of four potatoes from each hill were tested. In addition a representative sample for the hill was obtained by mixing equal parts by weight from each of the four potatoes. Four old potatoes from the 1945 crop were tested in the same way, namely, separately and a representative mixture of all four. The old potatoes did not represent separate hills.

The mixture of potatoes consistently gave an average value for the lot within  $\pm 5\%$ . Values for individual potatoes varied over such a wide range that average values seemed more useful for this survey than individual values. The greatest range occurred among the young tubers. One hill, in July, contained a potato that exceeded the average value by as much as 32%.

At the close of the season, in August 1947, 25 potatoes, all from the same lot, were tested in groups of five, that is five potatoes separately and mixed. The values for the 25 potatoes ranged from 39.9 to 57.4 mgm. of ascorbic acid per 100 gm. of dry weight of potato, or a range of about  $\pm 20\%$ . Each group of five potatoes gave average values, within  $\pm 5\%$ .

The values recorded in the following tables are average values. Four or more potatoes were mixed for each estimation, wherever possible. Occasionally, only two or three potatoes were available.

#### *Effect of Variety, Stage of Development, and Use of Top-killers on the Ascorbic Acid in the Potato Tuber*

Table I shows the effect on the ascorbic acid content produced by three variables—namely, variety, stage of development before maturity, and effect of use of top-killers.

During the period of growth of potato tubers, from the first week in August until the middle of September, eight varieties were tested at weekly intervals. These were grown on eight plots, where, as far as possible, growth and treatment conditions were the same. A ninth variety was tested during the latter part of this period. All the potatoes from each hill were counted, measured, and weighed. The values represent average values for hills.

A parallel series was tested to estimate the possible effect of top-killers on the ascorbic acid content of tubers.

The practice of top-killing has recently found widespread favor in the growing of seed potatoes. The tops are killed by sprays as the tubers begin to approach maturity. This permits earlier harvesting of the crop, which may take place about two weeks after application.

Three lots of potatoes from these 'killed' plants were tested through the month of September. Control plants were provided by the eight varieties already being tested at weekly intervals during growth. Tubers were tested for ascorbic acid within one to three days from the time of digging.

The sprays used with the different varieties have been indicated in the table.

TABLE I

ASCORBIC ACID IN THE POTATO SHOWING EFFECT OF VARIETY, STAGE OF DEVELOPMENT, AND USE OF TOP-KILLERS

Variety	Tops green							Tops killed by sprays					
	Aug. 8	Aug. 12	Aug. 15	Aug. 19	Aug. 23			Sept. 5	Sept. 10	Sept. 5	Sept. 10	Sept. 25	Top-killer
					Large	Med-ium	Small						
1. Bliss Triumph	210*	183	187	191	172	152		144	142	176	160	150	Dowspray Early
2. Chippewa	183	188	177	127	106		136	114	155	157 127	124	121	Handy Killer
3. Earlsaine	227	211	163	—		188		163	177	—	182	167	Dowspray
4. Green Mountain	161	135	172	113		131		127	133	126	129	101	Sinox
5. Houma	218	211	183	174	182	170		188	138	161	122	132	Dowspray
6. Irish Cobbler	195	150	173	170	155	128		127	136	136	130	140	Handy Killer
7. Katahdin	264	217	198	172	173		151	136	209	133	104	155	Dowspray Early
8. Sequoia	216	191	252	240	167		145	169	146	158	199	182	Dowspray Early
9. Sebago						178	152	182	230		172	120	Sinox

\* Ascorbic acid in mgm. per 100 gm. potato tissue (dry weight).

The first potatoes were dug on Aug. 8. Some were tested on that day. The remainder were stored at 40° F. until Aug. 12, when they were tested for ascorbic acid. After four days' storage there was either a slight decrease or no significant increase.

Similarly the potatoes of Aug. 15 were tested on Aug. 15 and Aug. 19. Again storage produced either a slight decrease or no significant change in ascorbic acid.

On Aug. 23, large, medium, and small potatoes from each variety were tested separately. A variation of  $\pm 12\%$  was noted within each variety, but no definite trend was observed at this date to correspond with variation in size.

On Sept. 5 and 10 two fresh lots of potatoes gave values for ascorbic acid lower than those recorded in August.

Tubers from top-killed plants were tested on Sept. 5, 10, and 25. No control set was available for those tested on Sept. 25.

Table I shows that the highest values for ascorbic acid were observed in August, followed by a steady decrease at various stages of growth until harvesting. There was no significant change in the values after storage for four days. No variation could be attributed to size of tuber at this stage of maturity.

The variation between varieties on the same day was as great as  $\pm 37\%$  (from 113 to 240 mgm. ascorbic acid per 100 gm. of dry weight of potato); but there was no constancy of position for any one variety. The Katahdin variety, which occupied first place on Aug. 8, later fell to fourth place. On Sept. 5 it was sixth while on Sept. 10 it was in second place.

In general through this six weeks' period the Katahdin, Houma, Sequoia, Earleine, and Sebago varieties ranked highest, the Bliss Triumph and Chippewa intermediate, and the Green Mountain and Irish Cobbler lowest. However, there was so much variation within each variety that it has been impossible to rank them in any definite order, to cover the whole period.

An average loss in ascorbic acid content of 25% was noted over this six weeks' period for all the varieties, with a range of loss from 15 to 37%.

Comparison of the ascorbic acid values in tubers from normal plants with those from plants killed by spraying showed no marked difference in the values, so that no effect could be traced to the treatment, at least during this time of observation.

#### *Effect of Different Fertilizers and of Storage on the Ascorbic Acid Content of Potatoes*

Table II shows the effects of fertilizers and of storage on the ascorbic acid content of potato tubers. It covers the period from July 1946 until the following May.

The different types of fertilizers and quantities are listed in the table. The Green Mountain variety of potato was used. Growth conditions were controlled so that as far as possible only the fertilizers varied.

Through July, August, and September average values were obtained for separate hills. From October to May the potatoes from different plots were stored in separate bins, at 40° F. By March, some of the tubers had started to sprout. The sprouts were removed from time to time, and analysis was done on tubers after removal of sprouts. The sprouts contained a considerable amount of ascorbic acid. No attempt has been made to explain the origin of this ascorbic acid nor to compare the total amounts in sprouted and unsprouted tubers.

During July and August the amount of ascorbic acid in the tubers was greatest, for all the fertilizers used. During September and October, while the tubers were still in the ground, the ascorbic acid decreased by as much as 25%. During storage the amount of ascorbic acid decreased until by the middle of December the value had fallen to about 50%. It continued to decrease until in May about 25 to 30% of the original amount remained, under these conditions of storage and regardless of the type of fertilizer.

During July and August the variation between different hills with the same fertilizer reached  $\pm 19\%$ . Variation between different fertilizers reached  $\pm 20\%$ .

Since the variation between different hills with the same fertilizer was as great as that between hills with different fertilizers no significant difference can be attributed to fertilizers.

During storage, when records of individual hills could no longer be obtained, monthly variations of plots with different fertilizers reached  $\pm 27\%$ . No single fertilizer gave consistently higher values than any other.

TABLE II

EFFECT OF DIFFERENT FERTILIZERS ON THE ASCORBIC ACID CONTENT OF  
POTATOES DURING GROWTH AND STORAGE

Plot No.	Fertilizer (per acre)	July 24	Aug. 2	Sept. 17	Oct. 8	Nov. 2	Nov. 12	Dec. 19	Mar. 18	May 27
1	1 ton 4-8-10 yearly from 1931	141* 155 149	182 136	145	144 153	127	98	78	83	47
2	$\frac{1}{2}$ ton 4-8-10 yearly from 1931	159 130	167 188 153	136	158 153	103	94	78	59	46
3	No fertilizer or manure since 1931	146 149 144	148 153 168 167	109	129	92	86	60	52	42
4	16 tons manure yearly since 1931	137 156 131 156	161 147 157	135	162	97	80	72	51	45
5	$\frac{1}{2}$ ton 4-8-10, 8 tons manure yearly since 1931	148 133 139	145 170 203	165	130	107	85	74	59	42
6-1	1 ton 4-8-10 basic, 3 year rotation		145 174 149	157	138	108	100	79	57	56
6-2	1 ton 4-8-10, 400 lb. 2-12-6				145	92	81	73	64	43
6-3	1 ton 4-8-10, 8 tons manure, new seeded land				142	97	95	79	63	44
6-4	1 ton 4-8-10, 8 tons manure, 500 lb. dolomite				143	81	86	68	62	45
6-5	1 ton 4-8-10, 500 lb. dolomite				152	95	82	75	65	48
6-6	No fertilizer or manure, 3 year rotation potatoes, grain, hay	134 150 155	158 137	135						

\* Ascorbic acid in mgm. per 100 gm. (dry weight).

This study shows that the type of fertilizer used does not have any significant effect on the amount of ascorbic acid present in potatoes during growth. The rate of decrease during storage is independent of the fertilizer used.

*Effects of Different Fertilisers in Different Localities on the Ascorbic Acid Content of Potatoes*

The effect of fertilizer was further investigated together with the effect of storage as shown in Table III.

TABLE III

ASCORBIC ACID CONTENT OF POTATOES GROWN WITH DIFFERENT  
FERTILIZERS IN DIFFERENT LOCALITIES

Locality	Fertilizer	Amount per acre, tons	Other treat- ment	Harvested	Ascorbic acid in mgm. per 100 gm. (dry wt.)			
					Dec.	Jan.	Mar.	May
Centerville	0- 8-10	1		Sept. 24	117	78	64	39
	2- 8-10	1		" 24	103	72	49	41
	4- 8-10	1		" 24	118	75	54	40
	6- 8-10	1		" 24	128	74	—	36
	4- 4-10	1		" 24	73	66	50	51
	4-12-10	1		" 24	86	62	56	38
	4- 8- 5	1		" 24	96	63	61	30
	4- 8-15	1		" 24	83	61	60	35
	4- 8-10	$\frac{1}{2}$		" 24	112	79	39	34
	4- 8-10	$\frac{1}{2}$		" 24	113	65	—	—
	4- 8-10	1		" 24	113	76	70	50
	None			" 24	83	81	71	48
Crockett	4- 8-10	1		Sept. 27	59	79	46	52
	4- 8-10	$\frac{1}{2}$		" 27	60	63	37	37
Guercherville	4- 8-10	1		Sept. 27	62	59	50	39
	4- 8-10	$\frac{1}{2}$		" 27	73	62	63	37
Mount Carmel	4- 8- 0			Sept. 20	62	67	48	38
	4- 8- 6			" 20	79	68	49	42
New Jerusalem	4- 8-10	1		Oct. 3	72	63	46	—
	4- 8-10	$\frac{1}{2}$		" 3	75	70	52	46
Saint Charles	None			Sept. 25	105	—	54	38
Saint Isidore	4- 0-10	$\frac{1}{2}$		Sept. 19	80	73	44	36
	4-16-10	$\frac{1}{2}$		" 19	58	69	52	48
Salisbury	4- 8-10	$\frac{1}{2}$		Sept. 21	72	75	55	—
	None		Manure	" 21	68	56	51	41
Sandwith	4-16-10			Oct. 2	96	61	63	44
	4- 0-10			" 2	108	98	55	32
Seigas	4-10- 0			Sept. 26	66	55	58	40
	4-10-12	$\frac{1}{2}$	Manure	" 26	54	69	45	37
	4-10- 6	$\frac{1}{2}$	Manure	" 26	59	77	56	37

These potatoes were grown on 30 plots throughout the province with known fertilizers. Soils and natural conditions varied with the localities. The Green Mountain variety was used for all plots.

The tubers were harvested from Sept. 24 to Oct. 3. They were stored in Fredericton under the same conditions as the other potatoes used in this survey. They were tested in December, January, March, and May.

*Centerville.*—In December the potatoes from these 12 plots varied from 73 to 128 mgm. of ascorbic acid per 100 gm. of dry weight. These values represented a variation of  $\pm 28\%$ . This variation is of the same order as the  $\pm 27\%$  found in Table II.

Different fertilizers did not produce any apparent effects on the ascorbic acid content of the tubers.

No single plot produced potatoes that gave consistently high or low values during the whole period of observation. Plot No. 4, which gave the highest values for ascorbic acid in December had fallen to eighth place in May. Plot No. 9, which occupied sixth place in December, rose to second in January, and fell to 10th place in March and in May.

*Crockett.*—The values from these two plots were low in December but increased in January. The one ton of fertilizer generally produced higher values than the half ton per acre.

*Guercherville.*—In December, January, and March the plot with one-half ton of fertilizer gave potatoes with more ascorbic acid than those from the plot with one ton of fertilizer.

*Mount Carmel.*—These two plots showed practically no variation with fertilizers. In December the values ranged from 62 to 79 mgm. per 100 gm., but in January, March, and May there was no significant difference.

*New Jerusalem.*—In these two plots the one ton of fertilizer per acre gave lower values for ascorbic acid than the half ton.

*Saint Charles.*—This plot, which had no fertilizer treatment, gave potatoes with a high ascorbic acid content in December. Later in the season, average values prevailed.

*Saint Isidore.*—These two plots gave average values with both kinds of fertilizer.

*Salisbury.*—One of these fields was treated with manure instead of fertilizer. In January, potatoes from the field treated with manure gave a low value.

*Sandwith.*—Values from these two plots showed much fluctuation but no no consistent effect was observed.

*Seigas.*—Two of these three plots had manure treatment as well as fertilizer. The values in December were relatively low.

For this part of the survey values were obtained for the ascorbic acid content of potatoes grown on 30 plots. The tubers were tested four times during storage.

No correlation was observed between values for ascorbic acid content and either fertilizer or locality.

In December the values ranged from 54 to 128 mgm. per 100 gm. of dry weight. In January they ranged from 55 to 98 mgm. per 100 gm. In March the values were from 37 to 71, and in May from 30 to 52 mgm. per 100 gm.

No single plot gave values that were consistently high or low during the whole period of observation.

From December to May the values decreased by about 50%.



*Value for Ascorbic Acid in Stem and Eye End of Tuber*

During October and November values were obtained for the ascorbic acid content in the stem and eye ends of some potatoes of the Green Mountain variety. The values are listed in Table IV.

TABLE IV  
ASCORBIC ACID IN THE STEM AND EYE ENDS OF GREEN MOUNTAIN POTATOES

Number	Date	Ascorbic acid in mgm. per 100 gm. (dry wt.)		Excess in eye end over stem end, %
		Stem end	Eye end	
1	Oct. 3	97	141	45
2	" 3	105	126	20
3	" 4	82	120	47
4	" 4	82	114	39
5	" 22	70	70	0
6	" 22	91	118	30
7	" 23	90	112	25
8	" 23	96	96	0
9	" 24	107	95	-11
10	" 24	70	101	45
11	" 25	81	109	35
12	" 25	82	74	-10
13	Nov. 4	101	82	-19
14	" 4	78	89	14
15	" 5	72	77	7
16	" 5	66	91	38
17	" 6	75	101	35
18	" 6	78	102	32
19	" 7	69	81	19
20	" 7	68	84	24
21	" 8	79	88	10
22	" 8	92	110	20
23	" 19	70	83	18
24	" 19	73	76	4
25	" 21	67	81	20
26	" 21	66	77	16

The eye ends were removed from the potatoes. They were chopped and thoroughly mixed. Similar representative samples were prepared from the stem ends of the same potatoes. Three to six tubers were included in each lot.

Twenty-six determinations were made. Twenty-one gave values for the eye end that were greater than those for the stem end, two were exactly the same, while only three had less ascorbic acid in the eye end than in the stem end.

The average excess of the ascorbic acid content in the eye end of the potato over the stem end was 20%. Statistical analysis of the values revealed that the excess of the eye end over the stem end was 15 mgm. per 100 gm. of dry weight. This excess was found to be significant.

This finding that the eye end of the potato contains a greater amount of ascorbic acid than the stem end is in agreement with results reported by Rolf (14) and by Murphy *et al.* (10).

It may be repeated here that for all the other determinations recorded in this paper, except those of Table IV, the potatoes were cut lengthwise, chopped, and mixed to obtain a thoroughly representative sample of whole tubers.

### Conclusions

The amount of ascorbic acid in potatoes grown in New Brunswick was determined during the period of growth and subsequent storage for the 1946-1947 season.

Values for ascorbic acid were highest in August. They were approximately 150 to 200 mgm. of ascorbic acid per 100 gm. of dry weight of potato tissue. They decreased to about 50% of that value in December, and to about 25% of the original value by the following May.

Variation between different varieties reached  $\pm 25\%$ . No variety maintained either a high or a low value during the whole season.

Variation within the Green Mountain variety reached  $\pm 27\%$ , when the potatoes were grown with different fertilizers. In different localities, the variation reached  $\pm 28\%$ . No single fertilizer or no single locality produced tubers that remained consistently high or low.

The eye end of the potato contained more ascorbic acid than the stem end contained. The excess was about 20%. On statistical analysis the excess of the eye end over the stem end was calculated to be 15 mgm. per 100 gm. of dry weight. This excess was found to be significant.

From these observations no correlation was observed between ascorbic acid content of potatoes and either fertilizer or variety.

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## AN EXPERIMENT TO MEASURE POTENTIAL EVAPO- TRANSPIRATION<sup>1</sup>

BY MARIE SANDERSON<sup>2</sup>

### Abstract

A method of obtaining daily values of the potential evaporating power of the atmosphere was used for the first time in Canada in 1947. The results verify Thornthwaite's formula for computing potential evapotranspiration in southern Ontario. The correlation between average runoff and computed water surplus for several watersheds in Ontario and Quebec is presented on a map.

The term potential evapotranspiration was introduced to climatological literature by C. W. Thornthwaite who defined it as the amount of water that will evaporate and transpire from a vegetation covered land surface while the root zone of the soil is well supplied with water. In his recent paper *An Approach Toward a Rational Classification of Climate* (3), Thornthwaite explains how he used existing experimental data to discover a relationship between potential evapotranspiration and other climatic factors for which there is abundant information. He devised a formula whereby water need can be computed for any station whose latitude is known and where temperature records are available. When the march of water need is compared with that of precipitation, periods of water surplus and water deficiency are revealed.

Thornthwaite used several kinds of information to test the reliability of his formula. Monthly figures of water use by irrigated crops were similar to the computed monthly water need, and average runoff for many eastern watersheds was the same as the average computed water surplus. In Canada average runoff was also compared with computed water surplus for watersheds containing weather stations (2). The correlation was surprisingly good, in view of the fact that the runoff is expressed in inches over the watershed and the water surplus is computed for a single point. The comparison between computed and measured runoff in southern Ontario and Quebec is presented in Fig. 1.

The best check on the formula and also a method of obtained daily values of potential evapotranspiration is to measure water need experimentally. Originally designed by Thornthwaite (4) for use in Mexico and called by the Mexicans an "evapotranspirometer", the apparatus was described by A. Contreras Arias in a recent Mexican Agricultural bulletin (1). During 1947 evapotranspirometers were installed in Mexico, in Toronto, Ont., and in Bridgeton, N.J.

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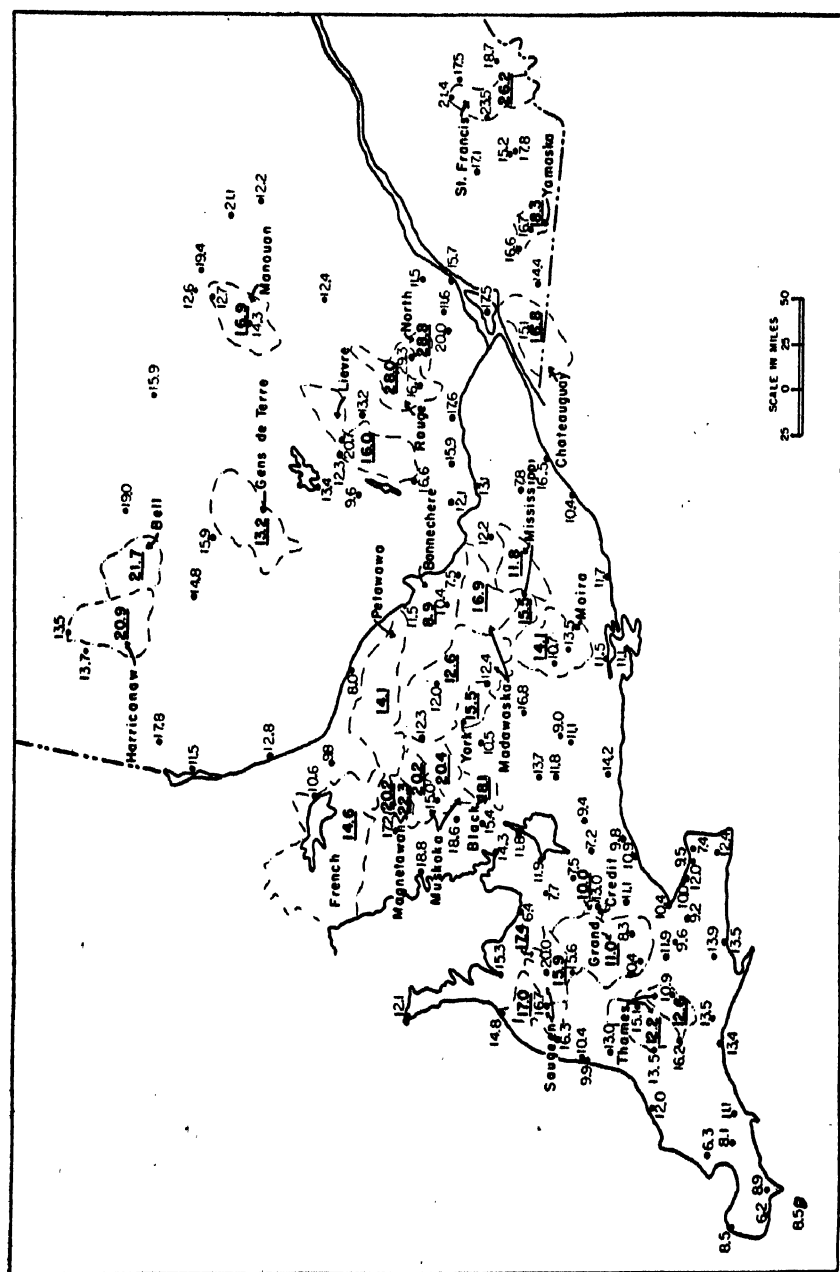


FIG. 1. Average annual water surplus in southern Ontario and Quebec (in inches). Light figures represent water surpluses computed by Thorntwaite method from meteorological data. Heavier, underlined figures represent average runoff as measured by Dominion Water and Power Bureau.

The present progress report describes a battery of evapotranspirometers installed by the Ontario Research Foundation at Toronto in June 1947, and gives all the observations obtained during the ensuing season.

### Description of Experiment

A diagrammatic sketch of the evapotranspirometer used by the Ontario Research Foundation (modified from the original Thornthwaite apparatus by L. J. Chapman) is shown in Fig. 2.

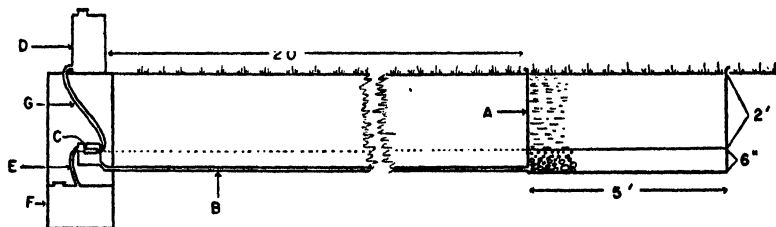


FIG. 2. Diagrammatic sketch of evapotranspirometer.

A. Galvanized iron tank (5 ft.  $\times$  5 ft.  $\times$  2½ ft.) buried in ground to within 2 in. of rim. Contains 6 in. of gravel, and filled with soil\* to ground level.

B. Underground iron pipe connects tank A with the water supply mechanism (on left).

C. Carburetor float valve to maintain water level in tank A constant at 2 ft. below surface.

D. Five gallon reservoir from which water is supplied to the system through tube G. Filled with water to a fixed point each day.

Amount of water necessary to fill reservoir is thus a measure of the daily water loss from tank A.

E, F. Mechanism to allow overflow. When rain occurs, and water level in A rises, intake from tank D is cut off by float valve; water reverses its flow, and empties, by means of copper tube E placed just above water level, into overflow tank F.

A distance of 20 ft. separates tank A from the measuring apparatus, so that the microclimate of the tank is little affected by this protuberance.

\* Soil used was a natural sandy loam with good capillarity, a moisture equivalent of 15.0 and mechanical analysis as follows:

Tank	% coarse sand	% fine sand	% silt	% clay
A	22	23	39	16
B	19	36	33	12
C	21	29	35	15
D	17	32	36	15

The principle is simple: to keep a grass-covered area, with natural microclimatic conditions, constantly supplied with water by subirrigation: and to measure the daily water use. Since the soil is kept constantly moist, it is assumed that the plants will use all of the water they want and that evapotranspiration will depend solely on atmospheric conditions. Four tanks were installed. Two of the tanks were planted with crested wheat, a drought resistant grass, and two with timothy, a grass believed to have a high moisture requirement.

The location chosen for the experiment was Mount Pleasant cemetery in North Toronto (latitude 49° 40' N., altitude 500 ft.). It was selected since it contained the most accessible, large, open area within a few miles of the Ontario Research Foundation. Fig. 3 shows the actual site, chosen because

of its accessible water supply, and fairly level ground. The site is open to the winds on the three sides, but there is a small wooded area about 100 ft. to the north. The immediate surroundings were sown to rye, which was cut periodically during the summer, providing an excellent meadowlike environment. The grass was clipped in the tanks and the surrounding plot every few weeks, to keep it about 2 to 4 in. high, and prevent it from going to seed. During dry periods, the grass in the surrounding plot was watered to ensure continued growth.

Readings were taken daily at 8.00 A.M. Eastern Standard Time of the following:

1. The amount of water (in liters) necessary to fill each intake tank.
2. The precipitation, if any, caught in the rain gauge (in millimeters).
3. The overflow (in liters), if any, collected in the overflow tank.
4. The maximum and minimum temperature for the preceding 24 hr.

Water entering the soil of the evapotranspirometer comes either from the intake tank or from precipitation. Water can leave the tank only by overflowing into the overflow tank, or by upward transfer as water vapor into the air. The latter is the evapotranspiration that may be obtained as a difference.

$$E = P + I - O$$

where  $E$  is evapotranspiration,  $P$  is precipitation,  $I$  is intake, and  $O$  is overflow.

### Primary Observations

The daily readings for the five months while the transpirometers were in operation are given in Table I. The surface of the soil in the tanks remained moist throughout the season, and it is certain that the vegetation had all the moisture that it required. Nevertheless, the overflow worked well; when rain occurred and the ground water level in the tank rose, water backed up through the pipe, closed the float valve cutting off the supply of water from the intake tank, and then overflowed into the overflow tank. The observations appear to be reliable, and indicate the daily water loss from a grass covered area under approximately natural conditions.

There was no significant difference in the daily water use by timothy and crested wheat grass. The water loss was the same for all four plots, even at the beginning of the season, when the timothy provided a dense lush cover and the crested wheat grass cover was sparse. There was reason to expect sizeable variations in the daily readings of the four tanks even after it became apparent that the species of grass and the amount of soil exposed made little difference. The soil, of course, was not identical in the tanks (Fig. 2) and capillarity could vary. Nevertheless, the variations were not great. In 75% of the readings uncomplicated by precipitation, the variation for the 24 hr. period was less than 1 liter or 0.44 mm. expressed as water loss from the surface of the tanks. For 35% of the time, the variation was less than 200 cc. or 0.09 mm.

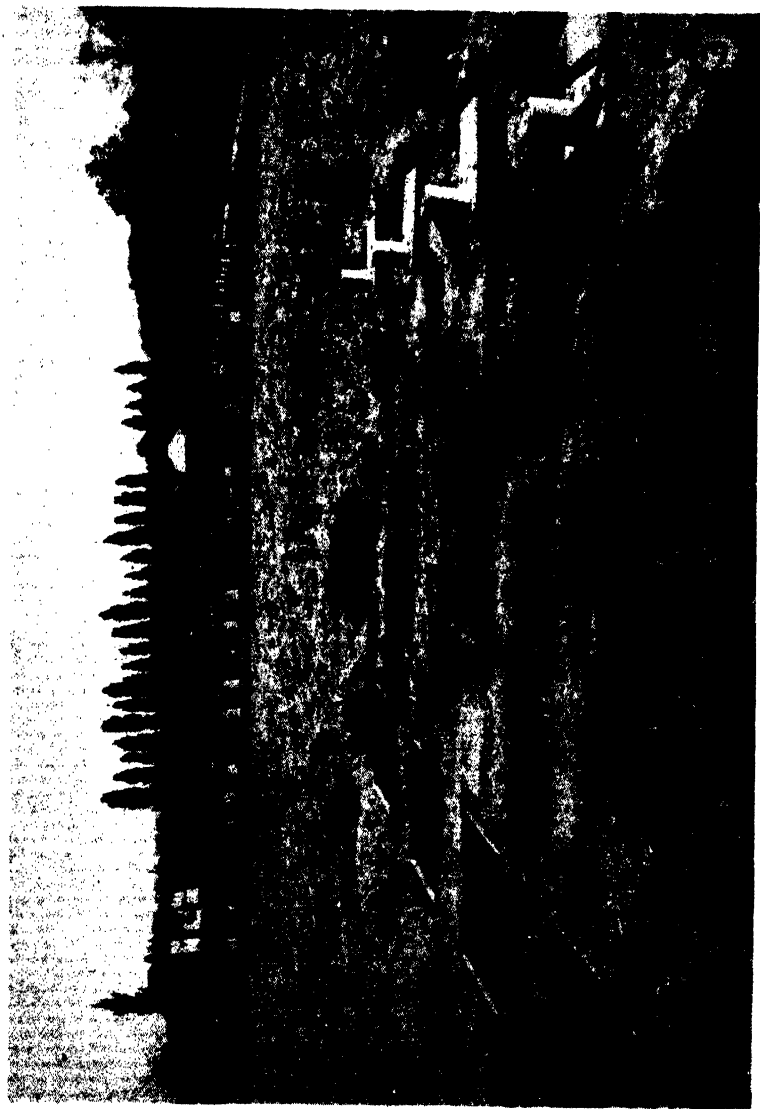


FIG. 3. Ontario Research Foundation potential evapotranspirometers, Mount Pleasant, Toronto, June, 1947. Transpirometer tanks at left, not yet seeded.





TABLE I

DAILY VALUES OF EVAPOTRANSPIRATION—TORONTO, 1947

Date	Temperature			P (mm.)	Water added (mm.)				Overflow (mm.)				Evapotranspiration (mm.)				
	Max.	Min.	Mn.		A	B	C	D	A	B	C	D	A	B	C	D	Com- puted
June 25	74.0	61.1	67.5	0.76	3.32	3.38	3.66	2.78	0.35	0.33	0.33	0.33	3.73	3.81	4.09	3.21	4.12
26	78.4	57.1	67.7		3.36	3.38	3.56	3.89					3.36	3.38	3.56	3.89	4.20
27	80.0	60.0	70.0		4.34	4.35	4.75	—					4.34	4.35	4.75	—	4.36
28	83.0	64.1	73.6		4.82	4.95	5.03	4.91					4.82	4.95	5.03	4.91	4.90
29	85.5	67.6	76.6		4.80	4.85	4.85	4.76					4.80	4.85	4.85	4.76	5.40
30	84.6	68.2	76.4		5.14	5.11	5.25	5.22					5.14	5.11	5.25	5.22	5.40
July 1	75.5	60.3	67.9		4.52	4.26	4.50	4.60					4.52	4.26	4.50	4.60	4.30
2	70.5	57.0	63.8		5.00	4.90	4.76	4.60					5.00	4.90	4.76	4.60	3.70
3	78.0	35.2	66.6		4.28	4.44	4.58	4.74					4.28	4.44	4.58	4.74	4.00
4	83.5	57.5	70.5		5.35	5.34	5.25	4.82					5.35	5.34	5.25	4.82	4.48
5	79.5	62.5	71.0	7.11	0.98	0.89	1.28	0.44	3.98	3.56	3.98	2.21	4.11	4.44	4.41	5.34	4.61
6	79.5	62.5	71.0	11.68	0.84	1.05	1.41	1.33	8.86	8.02	8.35	6.20	3.66	4.71	4.74	6.81	4.61
7	73.5	57.0	65.2		3.05	2.52	—	—					3.05	2.52	—	—	3.84
8	74.0	61.0	67.5		2.88	3.02	—	2.70					2.88	3.02	—	2.70	4.09
9	73.5	61.0	67.2		3.90	3.72	3.48	3.25					3.90	3.72	3.48	3.25	4.06
10	80.3	61.5	70.9	0.25	4.55	4.75	4.42	4.20					4.80	5.00	4.67	4.45	4.55
11	79.2	63.0	71.1		4.55	4.55	4.32	4.25					4.55	4.55	4.32	4.25	4.57
12	80.7	61.5	71.1	8.13	1.55	1.42	2.20	1.41	6.08	4.66	5.95	3.98	3.60	4.89	4.38	5.56	4.57
13	84.0	66.0	75.0		2.65	2.53	2.82	2.12					3.03	2.93	2.82	2.12	5.08
14	82.2	64.8	73.5	17.27	4.11	4.00	3.62	3.01	14.20	12.60	11.90	8.85	7.18	8.67	8.99	11.43	4.83
15	77.8	65.5	71.6	2.79	1.06	0.80	0.66	0.09	1.77	1.87	2.56	2.88	2.08	1.72	0.89	0	4.54
16	80.4	67.4	73.9		2.65	2.53	2.86	1.50					2.65	2.53	2.86	1.50	4.92
17	82.2	65.0	73.6	2.03	1.68	1.60	1.68	1.24	0.53	0.09	1.06	0.13	3.18	3.54	2.65	3.14	4.79
18	83.0	57.0	70.0	2.29	1.15	1.46	1.28	1.06	0.66	0	0.44	0.09	2.78	3.75	3.13	3.26	4.40
19	65.6	48.0	56.8		2.88	2.84	2.39	2.30					2.88	2.84	2.39	2.30	2.77
20	73.8	57.0	65.4	8.89	4.78	4.99	4.36	3.76	5.75	4.65	5.32	3.32	7.92	9.23	7.93	9.33	3.75
21	70.0	57.4	63.7		0.93	0.71	0.58	0.04					0.93	0.71	0.58	0.04	3.50
22	62.5	52.8	57.6	32.00	0.26	0.31	0.18	0.31	28.70	27.40	26.60	24.35	3.56	4.91	5.58	7.96	2.75
23	67.8	54.7	61.2		1.33	0.62	0.40	0.09					1.33	0.62	0.40	0.09	3.22
24	79.8	57.5	68.6		4.55	4.12	11.00	2.68	0.22	0.66	10.30	2.68	4.33	3.46	0.70	0	4.10
25	83.2	59.0	71.1		4.65	4.62	5.50	4.07					4.65	4.62	5.50	4.07	4.46
26	82.4	64.0	73.2		3.72	3.87	3.67	3.76					3.72	3.87	3.67	3.76	4.70
27	69.0	61.0	65.0	41.40	0	0	0	0	38.90	36.40	35.20	33.90	2.50	5.00	6.20	7.50	3.70
28	80.0	61.0	70.5		2.21	1.87	1.58	0.80					2.21	1.87	1.58	0.80	4.30
29	88.8	67.5	78.1		4.70	4.61	4.27	3.78					4.70	4.61	4.27	3.78	5.29
30	85.0	63.0	74.0		4.91	5.34	4.87	4.75					4.91	5.34	4.87	4.75	4.75
31	71.0	46.0	58.5		5.35	5.35	5.48	5.05					5.35	5.35	5.48	5.05	2.80
Aug. 1	67.0	47.0	57.0		4.86	5.02	4.76	4.83					4.86	5.02	4.76	4.83	2.69
2	74.1	54.2	64.1		4.07	4.13	4.00	4.25					4.07	4.13	4.00	4.25	3.54
3	80.8	59.0	69.9		4.60	4.76	4.48	4.60					4.60	4.76	4.48	4.60	4.26
4	82.8	60.2	71.5		5.32	5.20	5.30	5.45					5.32	5.20	5.30	5.45	4.36
5	84.8	63.5	74.1		4.78	5.29	4.72	5.05					4.78	5.29	4.72	5.05	4.72
6	87.2	62.6	74.9		4.37	4.50	5.07	4.83					4.37	4.50	5.07	4.83	4.80
7	86.9	66.0	76.4		4.74	4.98	4.76	5.14					4.74	4.98	4.76	5.14	5.04
8	83.0	65.5	74.3		2.61	2.91	2.82	3.21					2.61	2.91	2.82	3.21	4.64
9	81.0	59.2	70.1		4.38	—	4.45	4.60					4.38	—	4.45	4.60	4.17
10	81.0	64.0	72.5		5.68	—	5.67	5.53					5.68	—	5.67	5.53	4.37
11	85.6	64.2	74.9		3.54	—	4.03	4.00					3.54	—	4.03	4.00	4.72
12	93.2	71.0	82.1		3.54	—	3.46	3.67					3.54	—	3.46	3.67	5.55
13	93.0	73.0	83.0		4.25	—	4.43	4.56					4.25	—	4.43	4.56	5.73
14	91.5	69.5	80.5		1.88	—	1.72	2.04					1.88	—	1.72	2.04	5.38
15	77.5	62.2	69.9	5.59	0.89	—	0.79	0.53	1.79	—	0	0.44	4.69	—	6.38	5.68	4.10
16	77.8	59.0	68.4		4.45	—	4.52	4.31					4.45	—	4.52	4.31	3.88
17	78.0	66.0	72.0		2.78	—	3.13	3.28					2.78	—	3.13	3.28	4.20
18	89.5	69.0	79.3	10.67	3.52	—	3.62	3.54	8.05	—	7.85	4.20	6.14	—	6.44	10.01	5.18

TABLE I—Continued

DAILY VALUES OF EVAPOTRANSPIRATION—TORONTO, 1947—Continued

Date	Temperature			P (mm.)	Water added (mm.)				Overflow (mm.)				Evapotranspiration (mm.)				
	Max.	Min.	Mn.		A	B	C	D	A	B	C	D	A	B	C	D	Com- puted
Aug. 19	84.5	68.5	76.5	9.40	0	-	0	0	9.70	-	9.40	9.40	0.30	-	0	0	4.83
20	77.8	67.5	72.6		1.10	-	0.93	0.38					1.10	-	0.93	0.38	4.26
21	79.3	69.0	74.2		1.41	-	1.41	1.22					1.41	-	1.41	1.22	4.50
22	78.9	54.2	66.5		2.39	2.48	2.57	2.35					2.39	2.48	2.57	2.35	3.54
23	81.4	66.9	74.1		3.28	3.32	3.36	3.30					3.28	3.32	3.36	3.30	4.45
24	89.8	70.0	79.9	1.27	1.72	1.84	1.59	1.95					2.99	3.11	2.86	3.22	5.09
25	82.2	66.4	74.3	1.52	2.52	2.61	2.74	2.65					4.04	4.13	4.26	4.17	4.40
26	73.5	60.3	66.9	0.25	0.91	0.86	0.88	0.66					1.16	1.11	1.13	0.91	3.62
27	76.5	57.5	67.0		4.20	4.10	4.50	4.69					4.20	4.10	4.50	4.69	3.58
28	84.7	57.5	71.1		4.11	4.22	4.32	4.38					4.11	4.22	4.32	4.38	4.04
29	72.5	61.0	66.8	0.20	3.40	3.58	3.52	3.94					3.60	3.78	3.72	4.14	3.58
30	82.5	57.0	69.8	7.62	2.41	2.56	2.43	2.30	7.74	6.45	6.20	3.76	2.29	3.73	3.85	6.16	3.88
31	73.5	52.0	62.8		2.70	2.58	2.44	1.68					2.70	2.58	2.44	1.68	3.11
Sept. 1	71.5	60.0	65.8	2.29	2.39	2.39	2.28	2.17					4.68	4.68	4.57	4.46	3.41
2	79.0	57.8	68.4		3.37	3.23	3.38	2.84					3.37	3.23	3.38	2.84	3.64
3	81.3	57.0	69.1		3.54	3.85	4.23	4.20					3.54	3.85	4.23	4.20	3.74
4	84.2	59.6	71.9		4.52	4.08	4.24	4.35					4.52	4.08	4.24	4.35	4.04
5	75.8	59.6	67.7		2.88	3.02	2.77	-					2.88	3.02	2.77	-	3.54
6	82.0	62.5	72.2		3.83	3.94	3.85	-					3.83	3.94	3.85	-	4.00
7	87.1	61.5	74.3		3.38	3.42	3.47	-					3.38	3.42	3.47	-	4.22
8	74.8	59.6	67.2		3.74	3.86	3.67	-					3.74	3.86	3.67	-	3.43
9	83.7	69.2	76.4		3.36	3.60	3.62	-					3.36	3.60	3.62	-	4.49
10	85.9	71.3	78.6		3.06	3.29	3.67	-					3.06	3.29	3.67	-	4.71
11	85.2	70.0	77.6		2.26	2.31	2.44	-					2.26	2.31	2.44	-	4.56
12	82.8	66.2	74.5		2.26	2.05	2.39	-					2.26	2.05	2.39	-	4.10
13	81.0	60.2	70.6	0.76	1.04	0.84	1.06	-					1.80	1.50	1.82	-	3.68
14	80.0	65.0	72.5		3.41	3.15	3.34	-					3.41	3.15	3.34	-	3.88
15	77.6	49.8	63.7	4.83	0.88	0.84	1.06	-	3.94	2.30	2.87	-	1.77	3.37	3.02	-	2.94
16	66.5	48.0	57.2		2.39	2.04	2.34	-					2.39	2.04	2.34	-	2.29
17	75.4	53.0	64.2		2.70	2.66	2.61	2.30					2.70	2.66	2.61	2.30	3.02
18	78.5	66.0	72.2		2.34	2.38	2.57	2.66					2.34	2.38	2.57	2.66	3.82
19	77.5	50.6	64.0	7.11	0.67	0.55	0.64	1.99	7.00	6.70	6.70	-	0.78	0.96	1.05	-	3.00
20	71.5	55.0	63.2		0.75	0.49	0.62	2.34					0.75	0.49	0.62	2.34	2.86
21	78.8	53.0	65.9	33.53	0.84	0.71	0.75	0.97	28.70	26.60	26.90	-	5.67	7.64	7.38	-	3.26
22	51.9	33.0	42.4		1.04	0.51	0.49	0.13					1.04	0.51	0.49	0.13	0.81
23	57.2	40.4	48.8		1.95	1.78	1.78	1.68					1.95	1.78	1.78	1.68	1.41
24	68.7	37.0	52.8		0.44	2.14	1.99	2.17					0.44	2.14	1.99	2.17	1.82
25	54.0	32.8	43.4		2.78	2.39	2.30	2.54					2.78	2.39	2.30	2.54	0.90
26	53.5	33.0	43.2		2.21	2.21	2.05	2.44					2.21	2.21	2.05	2.44	0.90
27	52.0	31.5	41.8		2.05	2.14	2.01	2.44					2.05	2.14	2.01	2.44	0.80
28	57.9	47.2	52.6	1.52	1.55	1.59	1.59	1.90					3.07	3.11	3.11	3.42	1.68
29	57.0	33.4	45.2	0.76	0.35	0.24	0.18	0.04	0.60	0.80	1.02	0.22	0.51	0.20	0.08	0.58	0.99
30	47.9	30.5	39.2		1.48	1.35	1.46	1.53					1.48	1.35	1.46	1.53	0.47
Tank D omitted from record hereafter																	
Oct. 1	58.0	45.0	51.5		1.97	1.95	1.77						1.97	1.95	1.77		1.57
2	64.2	39.2	51.7		1.86	1.91	1.73						1.86	1.91	1.73		1.57
3	64.5	47.5	56.0		1.06	1.06	1.17						1.06	1.06	1.17		2.04
4	73.0	55.2	64.1		1.24	1.15	1.24						1.24	1.15	1.24		2.82
5	73.9	55.0	64.4		1.66	1.50	1.66						1.66	1.50	1.66		2.78
6	77.3	56.2	66.8		1.77	1.68	1.75						1.77	1.68	1.75		3.07
7	79.3	56.8	68.0		1.84	1.70	1.75						1.84	1.70	1.75		3.13
8	65.9	37.0	51.4		2.23	2.10	2.21						2.23	2.10	2.21		1.52
9	34.4	37.2	45.8		1.75	1.70	1.77						1.75	1.70	1.77		1.04
10	59.2	40.0	49.6		1.66	1.62	1.64						1.66	1.62	1.64		1.41
11	63.7	45.2	54.4		1.53	1.57	1.57						1.53	1.57	1.57		1.79

TABLE I—*Concluded*DAILY VALUES OF EVAPOTRANSPIRATION—TORONTO, 1947—*Concluded*

Date	Temperature			P (mm.)	Water added (mm.)				Overflow (mm.)				Evapotranspiration (mm.)				
	Max.	Min.	Mn.		A	B	C	D	A	B	C	D	A	B	C	D	Com- puted
Oct. 12	69.0	51.7	60.4		0.97	1.00	0.97						0.97	1.00	0.97		2.32
13	69.5	41.5	55.5	4.83	0.18	0.18	0.02		2.92	2.66	2.48		2.09	2.35	2.37		1.86
14	68.6	45.0	56.8		1.24	1.00	1.08						1.24	1.00	1.08		2.04
15	75.0	49.8	62.4		0.31	1.15	1.06						0.31	1.15	1.06		2.48
16	75.6	50.3	63.0		1.57	1.02	1.06						1.57	1.02	1.06		2.54
17	75.2	56.1	65.6		0.89	0.87	0.85						0.89	0.87	0.85		2.73
18	71.2	60.8	66.0	1.02	0.36	0.37	0.29		0.49	0.40	0.60		0.89	0.99	0.71		2.82
19	74.5	40.5	57.5		1.24	1.09	1.20						1.24	1.09	1.20		2.00
20	75.8	46.4	61.1		1.99	1.90	1.96						1.99	1.90	1.96		2.34
21	66.7	46.6	56.6		1.63	1.63	1.70						1.63	1.63	1.70		1.89
22	73.0	55.8	64.4		1.00	1.04	1.06						1.00	1.04	1.06		2.58
23	61.9	32.7	47.3		2.06	2.04	2.00						2.06	2.04	2.00		1.16
24	50.0	36.9	43.4		1.17	1.22	1.28						1.17	1.22	1.28		0.80
25	60.0	40.0	50.0		0.73	0.73	0.75						0.73	0.73	0.75		1.32
26	68.0	49.0	58.5		0.80	0.80	0.84						0.80	0.80	0.84		2.02
27	72.0	54.0	63.0	0.76	0.75	0.75	0.73						1.51	1.51	1.49		2.44
28	65.5	54.0	59.8	13.72	0.04	0.03	0.02						13.76	13.75	13.74		2.18
29	58.0	49.0	53.5		—	—	—						—	—	—		1.57
30	49.0	42.0	45.5	10.67	0	0	0		22.90	22.50	23.20		-12.23	-11.83	-12.53		0.95
31	48.2	32.2	40.2		0.47	0.20	0.29		0.47				0.47	0.20	0.29		0.52
Nov. 1	52.6	34.7	43.6		0.80	0.73	0.75						0.80	0.73	0.75		0.77
2	54.8	38.0	46.4		0.66	0.66	0.62						0.66	0.66	0.62		1.02
3	55.8	44.2	50.0		0.71	0.71	0.84						0.71	0.71	0.84		1.27
4	51.1	46.5	48.8	5.33	0	0	0						—	—	—		1.18
5	52.8	48.6	50.7	4.32	0	0	0		8.22	7.64	8.36		1.43	2.01	1.29		1.21
6	56.1	40.8	48.4	5.33	0.07	0.03	0.07		5.10	5.35	5.25		0.30	0.01	0.15		1.18
7	57.2	37.0	47.1	1.52	0.09	0.04	0.02						1.61	1.56	1.54		0.99
8	46.4	31.0	38.7		—	—	—						—	—	—		0.41
9	40.0	28.8	34.4		2.74	1.59	1.88		1.15	1.15	1.06		1.59	0.44	0.82		0.16
10	50.2	39.2	44.7		0.38	0.42	0.44						0.38	0.42	0.44		0.82
11	50.0	31.0	40.5	10.41	0.09	0.04	0.04		9.55	9.20	9.65		0.95	1.25	0.80		0.49
12	43.0	30.7	36.8		0.57	0.19	0.15						0.57	0.19	0.15		0.33
13	40.8	24.2	32.5		1.10	0.58	1.24						1.10	0.58	1.24		0
14	39.9	24.5	32.2		1.33	0.44	0.89						1.33	0.44	0.89		0

The most important observation was that the water losses measured by the evapotranspirometers were of the same order of magnitude as those computed from the Thornthwaite formula. The total monthly water losses for the period of observation are given in Table II.

The potential evapotranspiration computed from the formula seems to be consistently a little greater than the actual water loss. Fig. 4 shows accumulated measured potential evapotranspiration compared with the computed during a sample period—July 31 to Aug. 15. The daily measured water use differs from the computed, yet totals for the period shown in Fig. 4 are remarkably similar.

TABLE II  
TOTAL MONTHLY WATER LOSSES

	Tank A, Timothy		Tank B, Timothy		Tank C, Crested wheat		Tank D, Crested, wheat		Computed	
	Cm.	In.	Cm.	In.	Cm.	In.	Cm.	In.	Cm.	In.
June (6 days)	2.6	1.0	2.6	1.0	2.8	1.1	2.7	1.1	2.9	1.1
July	12.0	4.7	12.7	5.0	11.6	4.6	12.2	4.8	13.0	5.1
Aug.	11.0	4.3	(12.0)	4.7	11.6	4.6	12.2	4.8	13.4	5.3
Sept.	7.8	3.1	8.1	3.2	8.2	3.2	—	—	8.6	3.4
Oct.	4.1	1.6	4.0	1.6	4.0	1.6	—	—	6.1	2.4
Nov. (14 days)	1.1	0.4	0.9	0.4	1.0	0.4	—	—	1.0	0.4
Total	38.6	15.1	40.3	15.9	39.2	15.5	—	—	45.0	17.7

Measured and Computed Potential Evapotranspiration

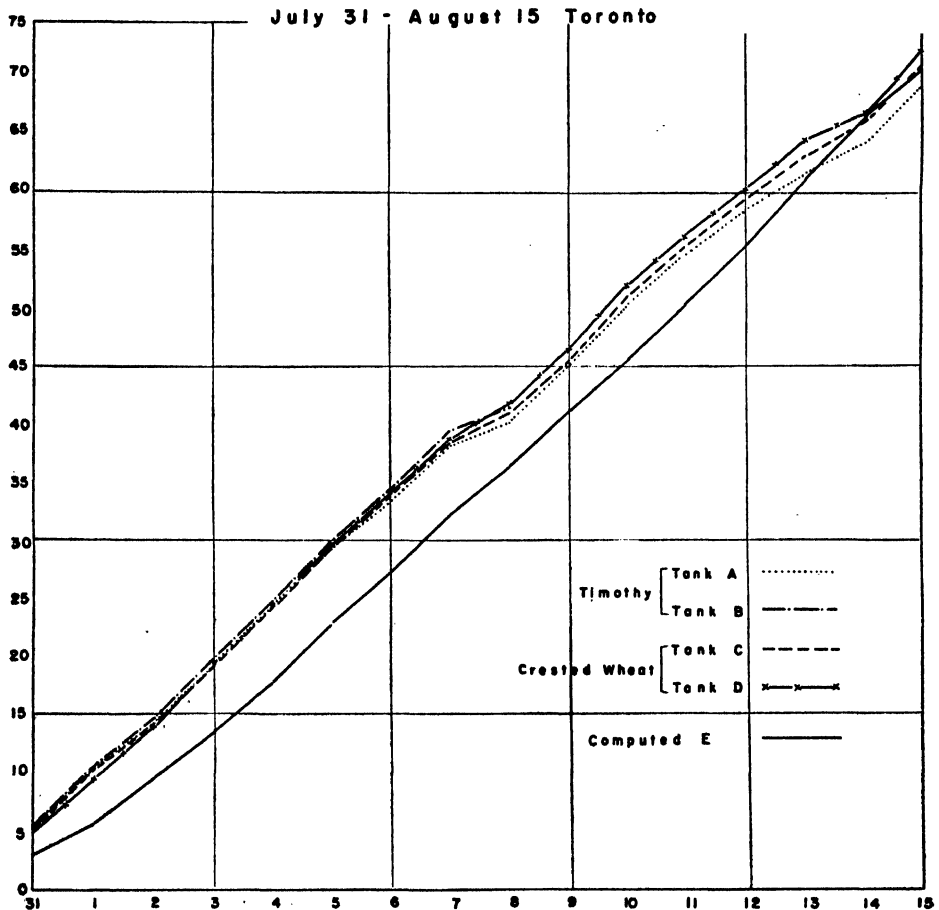


FIG. 4. Accumulated water loss (July 31 to Aug. 15) from timothy and crested wheat tanks, compared with accumulated computed water loss. Readings from tank B discontinued Aug. 8 because of leak.

### Results for Individual Days

It is interesting to compare the relation between measured and computed  $E$  with current weather, air masses, humidity, and wind. The following example illustrates different types of weather during the two weeks mentioned above and the resulting evapotranspiration.

On July 31 a polar continental air mass was moving into the Toronto area, the mean temperature was 58°, and the maximum 71° F. The wind was northwest at 16 m.p.h. the mean relative humidity was 64 and the measured ' $E$ ' of 5.1 to 5.5 was considerably higher than the computed  $E$  of 2.8 mm.

By Aug. 1, the cool air mass had covered Toronto, winds were north at 5 m.p.h. and the relative humidity reached a low of 55, with the maximum temperature 67 and the mean 57. Measured  $E$  varied from 4.8 to 5.0, again higher than the computed  $E$  of 2.7 mm.

By Aug. 2, the center of the high had moved to upstate New York, there was no wind at Toronto and relative humidity averaged 68. The mean and maximum air temperatures were higher, 64° and 74° F. respectively, and the measured  $E$  of 4.0 to 4.2 mm. was very little higher than the computed of 3.5 mm.

A different type of weather characterized Toronto from *Aug. 11 to 14*.

On Aug. 11, a tropical maritime high was centered just east of Toronto. There was no wind, a relative humidity of 78, a mean temperature of 75°, and a maximum of 86° F. The measured  $E$  was 3.5 to 4.0 mm., slightly lower than the computed  $E$  of 4.7 mm.

By Aug. 12, the high had moved over the Carolinas, hot gulf air was drawn north to Toronto and the maximum temperature reached 93°, while the mean for the day was 82°. Average wind velocity was 0, relative humidity 70, and the measured  $E$  was 3.5 to 3.7 mm., while the computed was 5.6 mm.

On Aug. 13 and 14, the high was stationary over the Carolinas and the same conditions prevailed in Toronto. Measured evapotranspiration on the 13th was 4.3 to 4.6 mm.; the computed 5.8. On the 14th the discrepancy between measured and computed evapotranspiration was the greatest. Measured  $E$  was only 1.7 to 2.0, the computed 5.4 mm.

From these two examples it appears that when tropical maritime air masses covered the station, the measured potential evapotranspiration was lower than the computed and when cooler continental, less humid air masses were present, the measured potential evapotranspiration was higher than the computed. On days with intermediate weather conditions, the measured water loss was similar to the computed. For example, on July 30, when the mean temperature was 74°, and average relative humidity 69°, the measured  $E$  was 4.75 — 4.9 mm. and the computed 4.75 mm.

### Probable Sources of Error

The amount of soil originally placed in the tanks was judged sufficient to allow settling but after several heavy rains in June, the soil settled 1 to 2 in.

below the general surface level. In this way the natural microclimate of the grasses may have been slightly modified.

When the daily readings were begun, it soon became obvious that after a rain, a certain unknown amount of water was held in the soil masses of each tank. It did not appear as surplus in the overflow tank, but supplied part of the moisture required for evapotranspiration in succeeding days. The amount varied with the four tanks and with different amounts of rainfall and resulted in uncertainty of the value of  $E$  for the day when precipitation occurred and at least one day following.

During the season, there was evidence that the precipitation sample from one 4.5 in. diameter rain gauge was not an adequate sample of the rain falling on the tanks. Because of the considerable weight given this figure in the computation, and the frequent rainy days (40 out of 140 during the period of observation), a larger rain sample should be taken.

### Conclusion

The present progress report on the Ontario Research Foundation evapotranspirometer experiments may prove helpful to others wishing to make similar measurements. The apparatus is inexpensive and simple to construct, yet it makes possible accurate daily readings of potential water need. In this respect it is a new and very important climatological instrument.

The first season of experiment indicates that there is no difference in the evapotranspiration from similar areas of different grasses, even though one has sparse and the other luxuriant growth.

The experiment has shown that the Thornthwaite formula gives values of potential water need of the right order of magnitude for Toronto. Computed and measured potential evapotranspiration for weekly or monthly periods were reassuringly similar. Daily values given by the formula are not accurate but with sufficient data, the present formula could be revised.

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## NOTES

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**PHOSPHORUS DEFICIENCY IN RELATION TO THE  
NITRATE REDUCTION TEST****Comment on a Paper by Isobel Dimmick**

The report by Dimmick (1) on the inability of certain types of bacteria to reduce nitrates when cultured on a medium containing calcium and their ability to reduce nitrate to nitrite in a similar medium containing either no calcium or an appreciably reduced amount, calls for comment. Dimmick considers that as in the preparation of the medium given in *The Manual of Methods for the Pure Culture Study of Bacteria* (2) the calcium is precipitated as an insoluble phosphate, the medium is then deficient in available phosphorus, not necessarily for growth of bacteria but possibly for the specific physiological reaction of the reduction of nitrate to nitrite. Further it is stated that there are many other media, used for a variety of purposes, that include in their composition phosphates together with comparatively large amounts of calcium, and that it is possible that bacteria growing in such media may suffer from phosphate deficiency.

Dimmick's interpretation of her results raises not only the question of the chemical composition of synthetic media but the usefulness and interpretation of differential tests in general. In the early days of bacteriology incorporation of different ions in a bacteriological medium was based largely on knowledge of the requirements of plants. Unfortunately often little regard was paid to the amounts of chemicals added and their compatibility. Worse examples exist than the incorporation of phosphate together with relatively large amounts of calcium under conditions that result in the precipitation of insoluble calcium phosphate. Some synthetic media, valued by their originators as being strictly reproducible, contain in addition to phosphate and calcium appreciable amounts of iron and magnesium; in these media a large proportion of the cations are precipitated as insoluble phosphates during preparation. Little attention appears to have been paid to the quantities of ingredients added in relation to the actual requirements of the bacteria and rarely any to the C/N ratio provided in the medium.

Dimmick's inference that the insoluble calcium phosphate produced in the 'Manual of Methods' nitrate medium limits the reduction of nitrate to nitrite by certain bacteria, by virtue of causing a phosphate deficiency in the medium, would have a wide application to bacteriology in general. It is therefore desirable to consider the phenomenon of nitrate reduction and to assess the importance of phosphate.

**NITRATE REDUCTION BY BACTERIA**

From the review of bacterial denitrification by Lloyd (3) and the work of Meiklejohn (4) the presence of nitrite in a nitrate medium can infer (a)



transient production of nitrite previous to further reduction i.e. a stage in the process of denitrification; (b) accumulation of nitrite due to conditions (acidity, etc.) preventing further reduction, or (c) a specific reaction in which nitrate is reduced only to nitrite, analogous to the specificity of *Nitrosomonas* reaction. Whereas there is little knowledge of reaction (c) Meiklejohn provides considerable information on (a) and (b). In brief, the results obtained with two species of denitrifying bacteria showed that although several organic compounds acted as substrates for growth not all would act as hydrogen donors for the denitrification reaction. With glucose and lactate as carbon sources denitrification proceeded to gaseous nitrogen; with lactose the end product was nitrite. It is clear from Meiklejohn's results that with a denitrifying culture the detection of nitrite in the medium depends not only on the period of incubation but on the specific carbon source used. It is of importance to note that Meiklejohn used a medium incorporating 0.01% calcium chloride and adjusted to pH 8.0 before steaming.

#### COMPARISON OF DIFFERENT SYNTHETIC NITRATE MEDIA

*The Reaction of the Medium.*—No precise details are provided by the Manual for the preparation of the synthetic liquid medium, nor by Dimmick for the preparation of the solid synthetic medium, particularly in regard to the adjustment of the pH value. In the first place liquid preparations of the Manual, Jensen's, and Dimmick's proposed medium were prepared. Part of each was sterilized by autoclaving in a pressure cooker for 15 min. at 15 lb. pressure, and part by steaming for one hour on three successive days. The pH values were determined by indicators.

TABLE I  
EFFECT OF HEATING ON THE pH VALUES OF SYNTHETIC NITRATE MEDIA

Medium	pH		
	Before heating	After steaming	After autoclaving
Manual	7.2	6.5	5.7
Jensen's	8.5	—	6.9
Dimmick's	8.4	7.3	6.8

Autoclaving produced an appreciable precipitate in Jensen's medium owing to the precipitation of magnesium phosphate. From Table I it can be seen that the acid reaction of the Manual medium, following sterilization, would be unfavorable for the growth of many types of bacteria.

*The Amount of Phosphate Precipitated.*—In order to test rather than speculate upon the amount of water-soluble phosphorus in the precipitate-free media, analyses were carried out on the clear supernatant liquor of both the Manual and Jensen's media.

The results (Table II) show that approximately half the phosphorus added to either Jensen's or the Manual medium is precipitated; in both media the amounts available for bacterial growth should be in excess of requirements.

**Growth Tests.**—The work of Dimmick was repeated. Solid media were prepared: (A) Manual, (B) Manual, sodium nitrate replacing potassium nitrate, (C) Manual, sodium chloride replacing calcium chloride, (D) Dimmick's

TABLE II  
AMOUNTS OF WATER-SOLUBLE PHOSPHORUS IN PRECIPITATE-FREE MEDIA

Medium	Phosphorus (mgm./liter P)	
	Added according to formula	Found in precipitate-free liquor after autoclaving
Jensen's Manual	178	88
	89	40

proposed medium, and (E) Manual adjusted to give a pH value of 7.2 after autoclaving. Colorimetric determinations of the pH values of the prepared media were (A) and (B), 5.8; (C) and (D), 6.7. The slopes of the agar media were streaked with cultures obtained from soil, mostly of the *Bacterium globiforme* type, and including *B. globiforme* Conn, and other similar types used in previous work (5). The amounts of growth were compared after incubation periods of three and six days at 20° C. and the test for nitrite applied on the latter occasion. There was little difference in the density of growth on the various media but in general (A) produced least growth and (E) was most favorable; the beneficial effect of the more neutral reaction of (E) was shown clearly. Of the 22 cultures examined 14 gave positive nitrite reactions to some degree in all media and two gave positive results in media (B), (C), and (D) only; one was positive in (A) only. Negative cultures were treated with zinc dust to ascertain whether the nitrate of the medium remained or had been reduced beyond the stage of nitrite. Of the five cultures that gave negative results in all media, four were found to have had no action on the nitrate; the fifth culture had reduced the nitrate beyond nitrite in media (A), (C), and (E). It was found that of the three cultures that gave anomalous results the negative reactions of two were explained by the reduction of nitrate beyond nitrite but the third culture had been unable to reduce the nitrate in media (A) and (E). Thus the only significant results of the test were that one culture could not reduce nitrate in media (B) and (D), and one in media (A) and (E) at the temperature and incubation period of the test.

A further test was carried out using 16 cultures of bacteria capable of multiplying in stored water and able to grow on a medium containing nitrogen

in inorganic form. Media (A) to (D) were employed. There was no obvious difference in the density of growth produced in the different media. The nitrite results with these cultures were not so clearly defined as was the case with the soil organisms. In some instances the test was only very faintly positive (0.2γ) and such reactions had to be considered as positive tests. Of the 16 cultures eight gave positive reactions in all media, including two cultures that reduced the nitrate beyond nitrite, and one culture was consistently negative. Of the remaining seven cultures four gave negative reaction on (A), four on (B), one on (C), and two on (D). Three of the four cultures giving negative tests on (A) were later found to give positive results when grown on (E), the medium of identical composition but adjusted to pH 7.2.

The most relevant point was that in some instances negative nitrite tests were due to the reduction of nitrate beyond nitrite, and hence in order to demonstrate the production of nitrite it is necessary to test the culture before reduction of all the nitrate is complete. To demonstrate this point several slopes of the various media were inoculated with a culture of *B. globiforme* Conn and nitrite tests were carried out after incubation for different periods at a temperature of 20° C. The phenomenon was common with other denitrifying cultures.

TABLE III

REDUCTION OF NITRATE IN DIFFERENT MEDIA BY *Bacterium globiforme* CONN

Medium	Period of incubation (days)				
	1	2	3	4	6
(A) Manual (CaCl <sub>2</sub> )	++	++	—	—	—
(C) Manual (NaCl)	+	+++	—	—	—
(D) Dimmick	++	+++	+++	—	—

Note: + = NO<sub>2</sub> positive; — = NO<sub>2</sub> negative.

## CONCLUSIONS

The results obtained in this work show that it is extremely unlikely that any inferiority of the Manual of Methods nitrate medium is due to phosphorus deficiency, either for the growth of bacteria, or for some physiological process affecting nitrate reduction. On the other hand there is strong evidence to suggest that inability to demonstrate the presence of nitrite in a culture of a nitrate-reducing bacterium is accounted for by either an acidic reaction of the medium or, in the case of denitrifying bacteria, by the fact that nitrate may have been reduced beyond nitrite. The importance of using a medium adjusted to the alkaline side of neutrality cannot be too strongly emphasized. In recording the ability or inability of an organism to reduce nitrate the experimental conditions should be stated in full.

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## ADAPTATION OF THE METHOD OF LOWRY AND LOPEZ TO THE ESTIMATION OF INORGANIC AND ORGANIC PHOSPHATE IN PLANT EXTRACTS<sup>1</sup>

By E. R. WAYGOOD<sup>2</sup>

### Abstract

The method of Lowry and Lopez using ascorbic acid as the reducing agent with the Fiske-Subbarow reagents at pH 4.0 is proposed for the estimation of phosphate in plant extracts. The method has been reinvestigated with respect to its adaptation to the Coleman Universal spectrophotometer, Model 14, to suitability of wave length, pH range, reagent concentration, rate of reaction, color stability, and the effect of silicate. The experiments, in general, confirm those of Lowry and Lopez. A wave length of 650 m $\mu$  has been employed. No blue color is formed in the reagent blank between a pH of 3.1 and 4.2; accordingly a pH of 4.0, maintained by a sodium acetate - acetic acid buffer, has been used. A concentration of 0.25% ammonium molybdate and 0.1% ascorbic acid has been shown to be optimum for maximum color development in concentrations of phosphate up to about 25  $\mu$ gm. P in the final volume. The reduction of phosphomolybdic acid is complete in about five minutes, and the method is characterized by a high degree of color stability. Silicate interferes with the reaction. Where the plant has a liberal silicate economy the cell wall material, with which the silicon is associated, must be removed from the extract. The method has been applied to the estimation of phosphate liberated from organic combination by hydrolysis for specified times in 1N hydrochloric acid. Total phosphate is estimated by wet ashing with 60% perchloric acid. Recovery tests of known amounts of inorganic phosphate and glucose-1-phosphate added to wheat leaf and seedling extracts give an absolute error within 2%, which is considered permissible. A balance sheet of phosphate fractions in the supernatant, residue, and whole juice of wheat leaves is discussed.

### Introduction

Methods for the determination of phosphate in plant and animal extracts are based on the formation of a blue coloration (molybdenum blue) by the action of a reducing agent on a phosphomolybdic acid complex. The method of Fiske and Subbarow (4) in which 1-amino-2-naphthol-4-sulphonic acid is used as the reductant has been widely adopted. Such modifications as have been applied to the method, in general, refer to the use of other and more suitable reducing agents (1, 5, 7, 8, 10). These investigators have employed stannous chloride (8), methyl-*p*-aminophenol sulphate (elon) (5, 7), 2,4-diaminophenol hydrochloride (amidol) (1), and ascorbic acid (10) as reducing agents in efforts to improve the color stability and acid tolerance of the

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method. Stannous chloride appears to be the most sensitive, but it has a very narrow range of acid tolerance and is quite unstable in dilute solution (5, 8). The sulphonic acid has a slightly wider range of acid tolerance (pH 0.5 to 0.6), but color development occurs apparently indefinitely (5, 7). Elon has proved to be more satisfactory, the sensitivity of the method is decreased, but its acid tolerance (pH 0.2 to 0.8) is even greater than with the sulphonic acid (7). Its solution is stable, and color stability is claimed after 30 to 60 min. (5, 7). Lowry and Lopez (10) used ascorbic acid as the reducing agent and showed that the acid safety limits for the reduction of phosphomolybdic acid were between a pH range of 0.4 to 0.9 and pH 2.8 to 4.6. They used the latter range in their estimations and showed that the higher pH conferred a greater degree of stability upon labile phosphate esters. Arney (2), after a preliminary separation of inorganic phosphate used the Fiske and Subbarow method for the estimation of phosphate cleaved from labile and resistant esters in barley extracts. Berenblum and Chain (3) modified the method using isobutyl alcohol as the reaction phase, in which they claimed the reduction of phosphomolybdic acid is more rapid and complete. Allen (1) found that their method was not applicable to extracts from certain higher plants and further modified the method for general use. Pons and Guthrie (11) have also investigated the validity of this method and adapted it for use with a spectrophotometer for the determination of plant materials high in protein.

Preliminary experiments were undertaken in this laboratory in order to determine which of these methods was best adapted to a study of phosphorylations and dephosphorylations in extracts and isolated enzyme systems of plants.

In the development of a colorimetric method, perhaps the main requirement is stability of color after the reaction has been completed. Since it is impossible to purify biological systems completely, conditions for the reaction to take place must be flexible and must allow for the uncontrollable variation in the natural buffers and interfering substances. Thus complicated methods and exacting procedures, with narrow ranges of acid tolerance, subject to color instability must be avoided if possible.

Initial tests conducted on the Fiske and Subbarow reagents using stannous chloride and 1-amino-2-naphthol-4-sulphonic acid confirmed the narrow range of acid tolerance at the low pH and the instability of the color developed. Elon was more satisfactory on our plant extracts, but the present author was unable to confirm the color stability claimed to be attained after 30 min. by Gomori (5) and after one hour by Holmes and Motzok (7) using this reagent.

The use of ascorbic acid as reducing agent was investigated and by virtue of its use it was found that the method conformed more rigidly to the requirements previously defined for a colorimetric method. The procedure of Berenblum and Chain (3) was tested, but the author was unable to obtain satisfactory results and hence it was decided to make a more detailed investigation

into the method of Lowry and Lopez (10) with a view to determine its applicability to plant extracts. Many determinations have since been made on extracts from parts of wheat, tomato, and onion plants using the adapted method and these have sufficed to confirm the validity of the method to be discussed.

## Experimental Results

### THE METHOD ON PURE SOLUTIONS OF PHOSPHATE

#### *Solutions Required*

1. Sodium acetate – acetic acid buffer: (0.025*N* and 0.1*N*), pH 4.0.
2. Ammonium molybdate: 5% aqueous solution, stored in an opaque, paraffin-wax lined glass stoppered bottle.
3. Ascorbic acid: 1% in acetate buffer (solution No. 1), stored under toluene in an opaque glass stoppered bottle at 4° C.
4. Potassium dihydrogen phosphate: stock solution, containing 1 mgm. P per ml. (2.195 gm. per 500 ml. water).
5. Solution No. 4 diluted 100 times, contains 10  $\mu$ gm. P per ml.

The ammonium molybdate solution was found to be quite satisfactory without acidification if stored under the prescribed conditions. The ascorbic acid solution is quite stable for at least four months, if not longer, providing a layer of toluene covers the solution and it is stored in a refrigerator.

#### *The Colorimeter*

Any suitable photoelectric colorimeter may be employed. In this study the method has been adapted to the Coleman Universal spectrophotometer, Model 14, using 14–303 cuvettes, matched to  $\frac{1}{2}$ % transmission. However the method has been used successfully with the Klett-Summerson photoelectric colorimeter with a No. 69 (690*mμ*) filter in position.

#### *Procedure*

Transfer 1 ml. of solution No. 5 (10  $\mu$ gm. P per ml.) to a 10 ml. volumetric flask. Add 2 ml. of acetate buffer (pH 4.0, solution No. 1) and 0.5 ml. of ammonium molybdate (solution No. 2). Shake well. Add 1 ml. of ascorbic acid (solution No. 3), make to volume with distilled water, and after vigorous shaking, allow to stand for at least five minutes. At the same time a blank should be made using distilled water. After five or more minutes transfer the contents of each flask to the matched cuvettes and place in the spectrophotometer set at a wave length of 650 *mμ*. Adjust the galvanometer so that the blank reads 100% transmittance or zero optical density ( $D = -\log T$ ) and record the percentage transmittance when the phosphate solution is moved into the beam of light. At this wave length setting, the concentration of phosphorus, as will be seen later, is inversely proportional to the logarithm of the percentage transmittance (i.e.  $C = -k \log T$ ) and this reading will constitute a calibration factor for the instrument from zero concentration (100% *T*) up to and including a concentration of 10  $\mu$ gm. P measured.



An accurate calibration curve may be prepared by using various concentrations of phosphorus and plotting concentration against the logarithm of the percentage transmittance in the manner shown in Fig. 1.

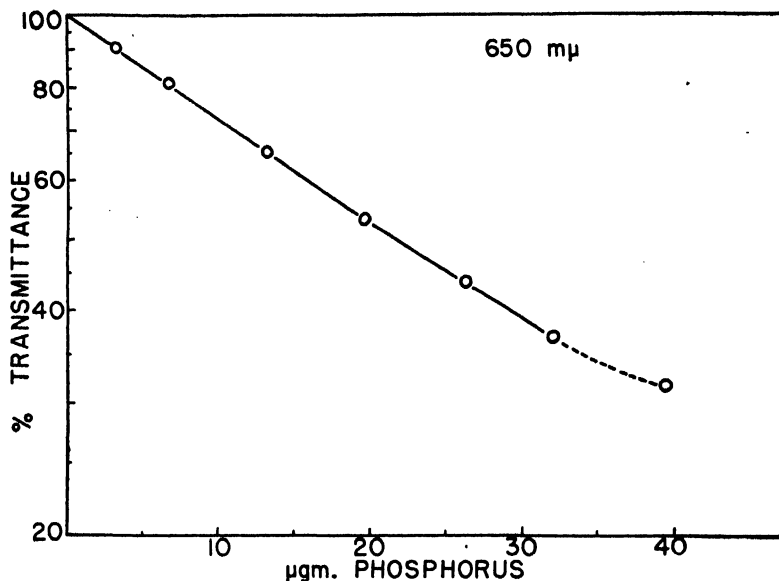


FIG. 1. Calibration curve of phosphorus using the Coleman Universal Spectrophotometer, Model 14, and showing close conformity to Beer's law ( $C = -k \log_{10} T$ ) at 650  $m\mu$  and pH 4.0. Dotted line, nonconformity to Beer's law at this concentration of phosphorus.

#### *Absorption Spectrum of the Reduced Phosphomolybdic Acid Complex*

In order to determine that the wave length of light should be so chosen that the relation between concentration and transmittance conforms to Beer's law ( $C = -k \log T$ ; Fig. 1) the absorption spectrum of the phosphomolybdic acid complex was followed over a range from 325  $m\mu$  to 825  $m\mu$ . Various concentrations of phosphorus were compared to the reagent blank set at 100% transmittance. The data are graphically represented in Fig. 2. The absorption spectrum of this blank compared to distilled water at 100% transmittance is also shown. The blank itself shows well defined absorption bands in the regions 325  $m\mu$  to 450  $m\mu$  and past 700  $m\mu$ , therefore these parts of the spectrum must be avoided for the colorimetric determination, since it is advisable to reduce to a minimum the absorption due to the reagents and their impurities. Analysis of the data show that Beer's law holds over the range from 500  $m\mu$  to 675  $m\mu$ , hence it was decided to use the wave length 650  $m\mu$ , which tends towards increasing the sensitivity of the method. The calibration curve at this wave length is shown in Fig. 1. It will be observed that there are three parts to this curve. The first up to 20  $\mu\text{gm. P}$ , the second from 20  $\mu\text{gm. P}$  to about 30  $\mu\text{gm. P}$ , and the third above 30  $\mu\text{gm. P}$ .

Deviation from Beer's law occurs at relatively high concentrations i.e. 20  $\mu\text{gm. P}$  to 30  $\mu\text{gm. P}$ , but the deviation is not sufficient to invalidate the

determinations in this region. Beyond a concentration of about 30  $\mu\text{gm. P}$  the deviation is much greater and determination in this region could not be relied upon.

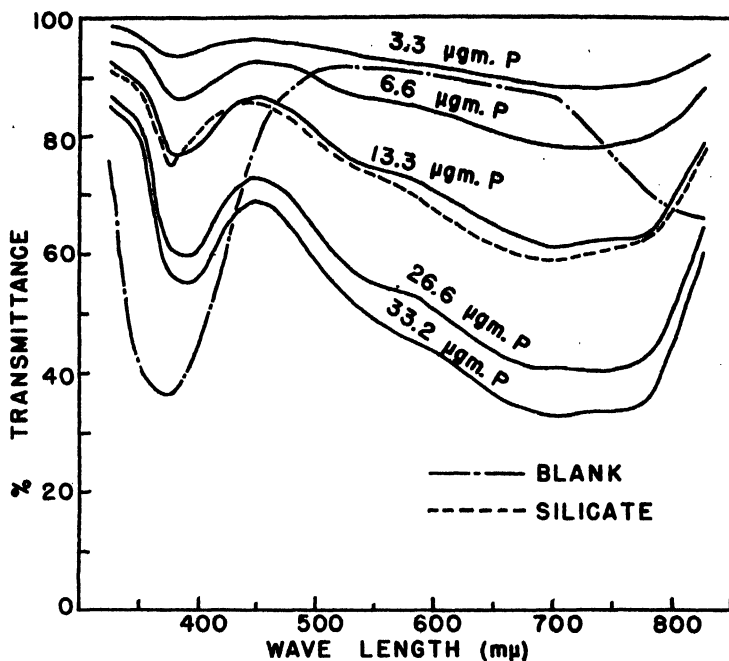


FIG. 2. Absorption spectra of reduced phosphomolybdic acid at pH 4.0 and various levels of phosphorus. Absorption spectra of the reagent blank and a molybdisilicic acid complex ( $120 \mu\text{gm. Si}$ ) at pH 4.0.

#### *Effect of Acidity on the Reduction of Molybdic Acid*

Molybdic acid as well as phosphomolybdic is reduced to molybdenum blue at certain pH values. The reduction of molybdic acid is determined by following the color development in the reagent blank at various pH levels. That region of the pH scale wherein no color develops in the blank compared to the blank at pH 4.0 is suitable for the reduction of phosphomolybdic acid. Accordingly the pH of the reagent blank was adjusted to a series of values between pH 0.5 and pH 4.5 by the addition of 10*N* sulphuric acid or 1*N* sodium acetate to the buffer solution No. 1. After readings had been taken of the absorption of the solutions, measured by optical density, the pH of each solution was measured accurately. A graph indicating the relation between pH and the reduction of molybdic acid compared to zero reduction at pH 4.0 is shown in Fig. 3. Reduction of molybdic acid occurs in the range from pH 1.0 to pH 3.1, and since the color was developing continually except in the blank, a specified time of 15 min. was chosen to record the transmittance of the solution. In the pH ranges where no absorption occurred when referred to the reagent blank at pH 4.0, readings were taken after five minutes, by

which time the optical density was stable. If various concentrations of phosphorus were admitted, the color development was proportionately higher in the pH range where no blue color developed in the blank, and considerably higher in the range pH 1.0 to pH 3.1, the latter indicating the combined

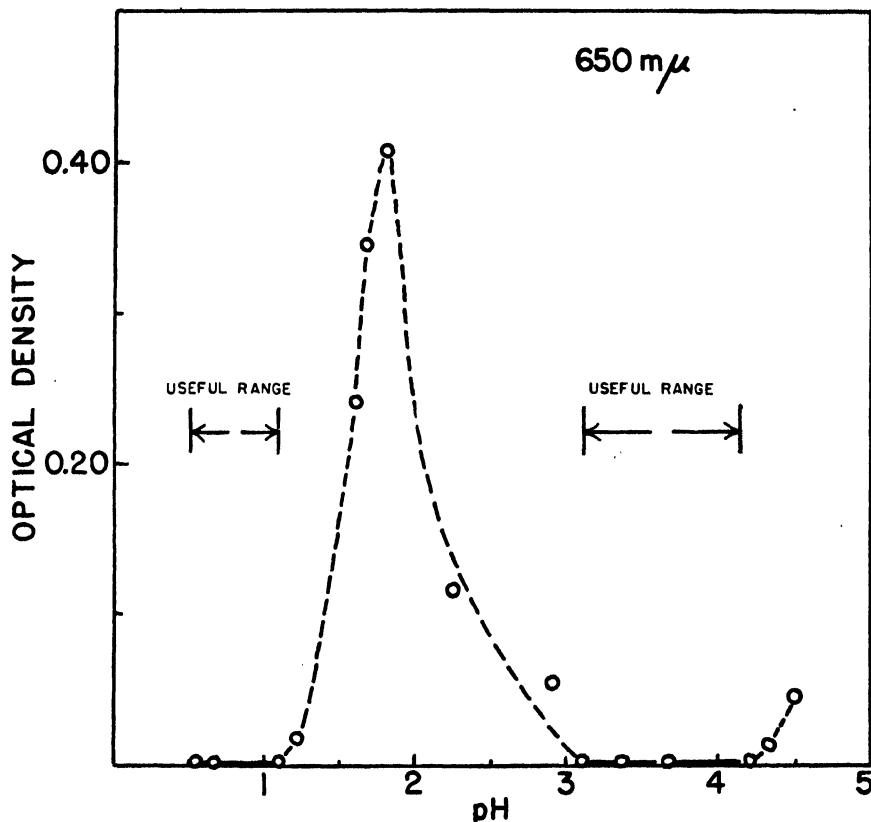


FIG. 3. Color development in the reagent blank at various levels of pH, showing the range where molybdic acid is itself reduced (650 mμ).

reduction of phosphomolybdic and molybdic acid, while the former indicates a separation of the two processes, phosphomolybdic acid being the only complex reduced. The data confirm the observations of Lowry and Lopez (10) as to the effect of pH. Of the two useful pH ranges for phosphorus determination, the higher one (pH 3.1 to pH 4.2) is chosen for two reasons. Firstly, the higher pH confers more stability on labile phosphate esters (10) and secondly the wide range confers a greater acid tolerance upon the method. A sodium acetate - acetic acid buffer, pH 4.0 (solution No. 1), has been found to be satisfactory. However in some cases, especially in the estimation of total phosphorus ( $P_t$ ) after the wet ashing procedure (see later), it has been found more convenient to use a stronger buffer solution made by acidifying 500 ml. of 1*N* sodium acetate with about 110 ml. of glacial acetic acid to pH 4.0.

*Effect of the Concentration of Ammonium Molybdate and Ascorbic Acid on the Reduction of Phosphomolybdic Acid*

Lowry and Lopez (10) found that the lowest permissible molybdate concentration for full color development was 0.05%. It is the present author's opinion that this concentration verges precariously on the lower safety limits for full color development. The optical density of phosphate solutions containing various concentrations of ammonium molybdate has been plotted against molybdate concentration compared to the reagent blank (0.25% ammonium molybdate) set at zero (Fig. 4). Maximum color development occurs at a concentration of ammonium molybdate over 0.125%; thereafter a

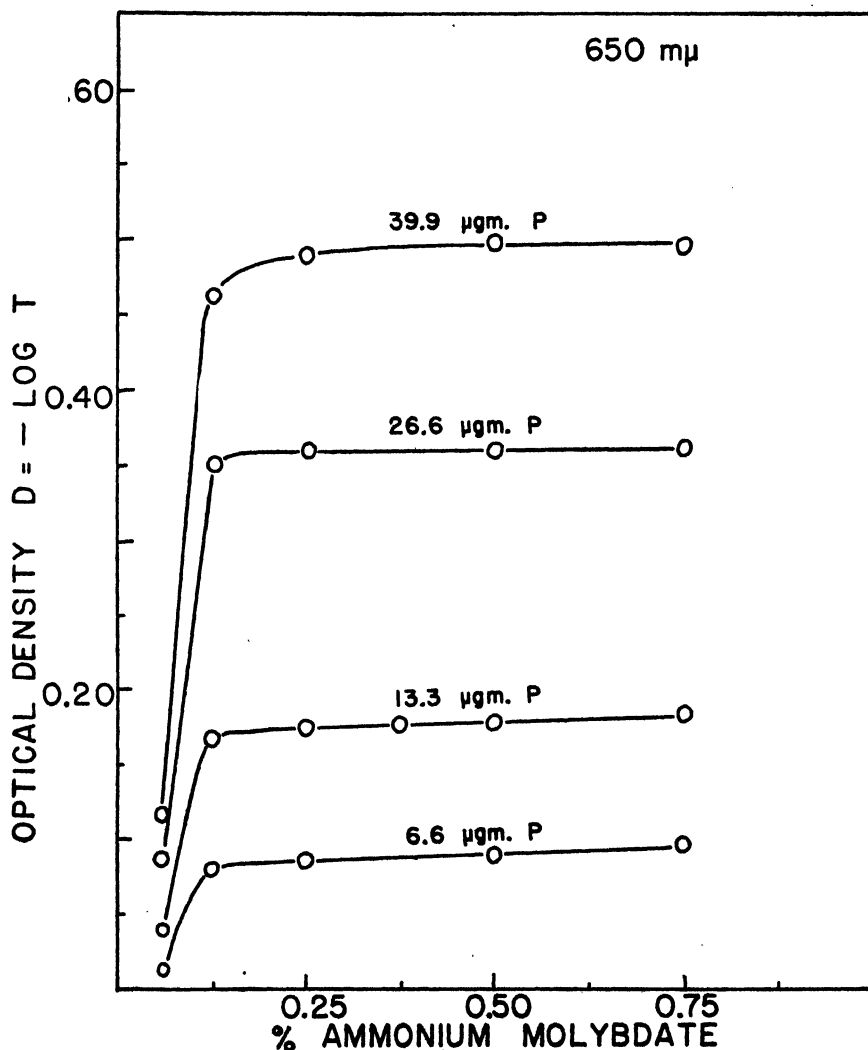


FIG. 4. Effect of the concentration of ammonium molybdate on color development at four levels of phosphorus (650 mμ, pH 4.0).

slight increase occurs in the two lower concentrations of phosphorus. At a level of 39.9  $\mu\text{gm. P}$  maximum color development occurs beyond a concentration of 0.25% ammonium molybdate and may account for the deviation from Beer's law at this concentration of phosphorus (Fig. 1). A value of 0.25% ammonium molybdate in the final volume was chosen as the optimum concentration since it is safely past the limiting values of ammonium molybdate for full color development. This concentration is equivalent to 0.5 ml. of 5% molybdate in 10 ml. of the final volume. If difficulties are encountered in the splitting of labile phosphate esters, e.g. acetyl phosphate, at this concentration of ammonium molybdate then a decrease to 0.125% would be permissible providing the concentration of phosphate is kept low.

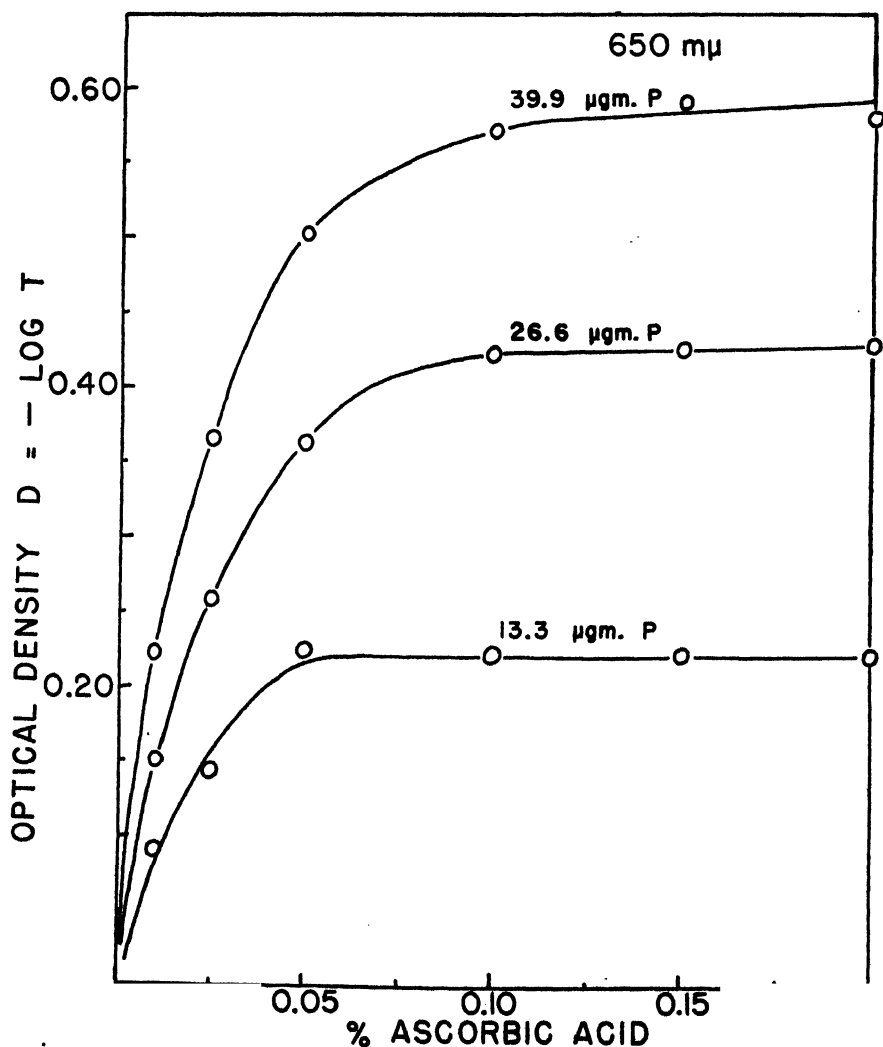


FIG. 5. Effect of the concentration of ascorbic acid on color development at three levels of phosphorus (650  $m\mu$ , pH 4.0.)

The ascorbic acid functions to reduce the phosphomolybdic acid complex to what is called "molybdenum blue". Color development in phosphate solutions at various concentrations of ascorbic acid in the final 10 ml. volume has been plotted against ascorbic acid concentration in Fig. 5. Maximum color development occurs in the two lower concentrations of phosphorus at 0.10%, thereafter no significant increase is brought about by increasing the ascorbic acid concentration. The highest concentration of phosphate is outside the range of the method. In this experiment the transmittance of each solution was compared against a distilled water blank set at 100% transmittance, thus allowing each curve to be extrapolated to the common graphical origin. A concentration of 0.10%, equivalent to 1 ml. of 1% ascorbic acid in 10 ml. of the final volume, was chosen as the optimum concentration required for the method.

#### *Rate of Reduction of Phosphomolybdic Acid*

Prior to ascorbic acid being used as a reducing agent in this reaction the time taken for complete reduction and uniform color development was of the order 30 to 60 min. and even then color instability was observed (5, 7). With ascorbic acid as the reducing agent, under the conditions prescribed, the reaction is complete in approximately five minutes, but the most outstanding feature is the high degree of color stability. Progress curves of the reaction are shown in Fig. 6 at three levels of phosphorus of which the highest is outside the range of the method and has been included as a study of an extreme case. The lowest level of phosphorus (10  $\mu$ gm. P) in this case was compared against the reagent blank, while the other two (26.6  $\mu$ gm. and 39.9  $\mu$ gm. P) were compared against distilled water set at zero optical density, hence the differing points of origin of these curves. Readings were taken for one hour, but have not been included owing to the insignificant variation from the equilibrium state achieved at three to six and one-half minutes at the three levels of phosphorus.

Earlier experiments showed that ammonium molybdate, rather than retarding the rate of color development (10), increased it, and some data on this point, using the Klett-Summerson photoelectric colorimeter, are assembled in Table I.

At the concentration of 0.25% ammonium molybdate, completion of the reaction was observed in approximately three-quarters of the time taken for a solution containing 0.5% ammonium molybdate and one-half the quantity of phosphorus. Fig. 6 shows that with increasing phosphate concentration under standard conditions the time for completion of the reaction is increased. Therefore one must conclude from the data in Table I that sufficiently high concentrations of ammonium molybdate depress the rate of reaction, although not affecting ultimate color development (cf. Fig. 4). This is further justification for maintaining the concentration of ammonium molybdate as low as permissible. Furthermore the presence of excess molybdate provides corresponding opportunity for its reduction to molybdenum blue.

### *The Interference Due to Silicates*

Both silicate and arsenate give rise to heteropoly compounds with molybdates which can be reduced to molybdenum blue (13). The latter would rarely be a significant interfering substance in plant extracts. However those plants

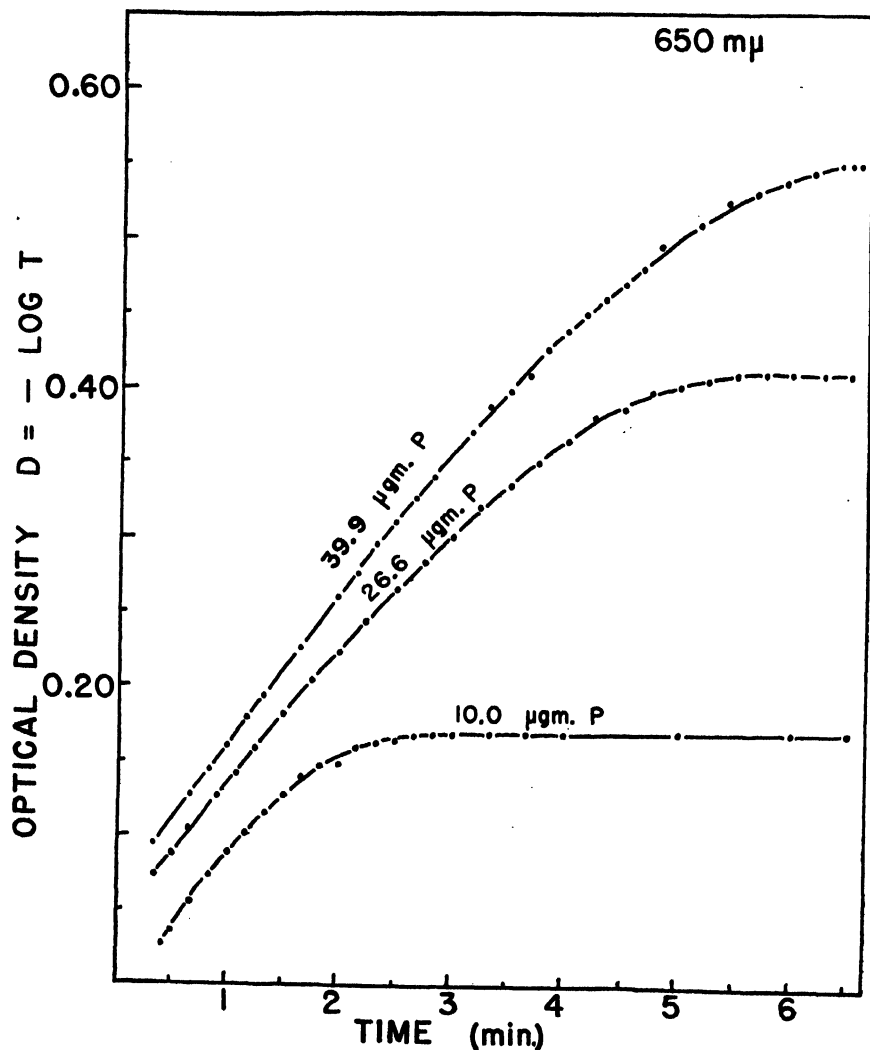


FIG. 6. Progress curves of the reduction of phosphomolybdic acid by ascorbic acid at pH 4.0 and 650 mμ, showing the rapid rate of reaction and uniform color developed.

having a liberal silicate economy might possibly present a serious problem in the determination of phosphorus. Woods and Mellon (13) using chlorostannous acid allow a permissible amount of 10 p.p.m. silicon (10 μgm. per ml.) to give an error within 2% phosphorus. A solution of sodium silicate ( $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ ) was made up containing 240 μgm. Si per ml. Of this solution

TABLE I

EFFECT OF CONCENTRATION OF AMMONIUM MOLYBDATE ON THE RATE OF REDUCTION OF PHOSPHOMOLYBDIC ACID

10 $\mu$ gm. phosphorus, 0.5% molybdate		20 $\mu$ gm. phosphorus, 0.25% molybdate	
Time, min.	Apparent P, $\mu$ gm.	Time, min.	Apparent P, $\mu$ gm.
0.75	2.2	0.33	2.1
1.00	4.1	0.58	3.9
1.50	5.8	0.75	6.0
1.66	6.8	1.00	9.9
1.92	7.8	1.33	11.7
2.25	8.5	1.58	13.4
2.75	9.3	1.75	15.2
3.00	9.7	1.92	17.5
3.25	9.8	2.01	19.0
4.00	9.9	2.25	20.5
4.25	9.9	2.33	20.6
4.50	9.9	2.40	20.7
5.00	9.9	3.00	20.7

0.5 ml. (120  $\mu$ gm. Si) was treated as in the procedure for phosphorus determination and the absorption spectrum followed over the wave length range as shown in Fig. 2. The absorption spectrum almost parallels that of phosphomolybdic acid. If the color development conforms to Beer's law at 650 m $\mu$  then approximately 1/10th (cf. Woods and Mellon (13) ) of this concentration (i.e. 12  $\mu$ gm. Si) would give an error within 2% in a total of 10  $\mu$ gm. phosphorus. Using the normal dilutions for the determination of phosphate in plant extracts, a transmittance of the order produced by 120  $\mu$ gm. Si would be equivalent to 0.6 gm. per 100 ml. of extract and the permissible amount would be 1/10th or 0.06 gm. per 100 ml. of extract. Latshaw and Miller (9) found a concentration of 2.5% silicon in the dry matter of whole corn leaves. Assuming 90% water content this value would be equivalent to 0.25 gm. per 100 gm. fresh leaves, a value four times as great as that permissible. However it is generally agreed that silicon impregnates the cell walls of plants. Hence procedures that extract juices and the more active metabolic constituents, eliminating cell wall material, would be only slightly affected by the presence of this element. In so far as procedures in this laboratory are concerned, the cell wall material is completely removed by coarse filtering and centrifugation and it is assumed that soluble silicates are not in sufficient proportion to interfere seriously with phosphate determinations in these isolated systems.

#### THE METHOD ON PLANT EXTRACTS AND ITS APPLICATION TO THE DETERMINATION OF ORGANIC PHOSPHATE COMBINATIONS

Having prescribed the conditions for the estimation of inorganic phosphorus in pure solutions, the method is now considered as to its suitability for the estimation of inorganic and organic combinations of phosphorus in plant extracts. Determinations of inorganic and organic phosphorus may be made



on the fresh or deproteinized juice. In the latter case ice cold 7.5% trichloroacetic acid is added to the juice in the proportion 5 : 1. The centrifuged extract is then brought to pH 4.0 by 1*N* sodium acetate and stored in the refrigerator. This procedure results in a solution that requires no further clarification for phosphorus determination.

If investigations are to be made on fresh juice, the juice is filtered through cheesecloth and centrifuged for a few minutes to remove cell debris and other readily precipitated material. The extract may be diluted with water or an appropriate phosphate or other buffer. In this manner an artificial phosphorus nutrition may be set up. The extract at this stage contains material, mostly chlorophyllous and some proteinaceous, that will separate on prolonged, centrifugation, or standing in the cold. If it is desired to separate this material a slight lowering of the pH, e.g. in wheat from pH 5.6 to 4.5, will precipitate such materials readily. The extract now contains proteins, inorganic phosphate, and a small amount of organic phosphate in labile combination, which declines to insignificance if allowed to stand without deproteinization. Fresh extracts are deproteinized at the time of determination.

#### *Inorganic Phosphorus ( $P_i$ )*

Transfer an aliquot, usually 1 ml., of the extract containing about 500  $\mu\text{gm.}$  P per ml. to a 50 ml. volumetric flask. Deproteinize with 5 ml. of cold 7.5% trichloroacetic acid and immediately bring the extract to pH 4.0 by an addition of 1*N* sodium acetate. Make to volume with distilled water, centrifuge for five minutes, and use 1 ml. for the estimation of phosphorus described previously for pure solutions. Recovery tests are shown in Table II.

TABLE II  
RECOVERY OF INORGANIC PHOSPHATE FROM WHEAT LEAF EXTRACT

Test material	$P_i$				$P_t$			
	$\mu\text{gm./ml.}$		%		$\mu\text{gm./ml.}$		%	
	Calc.	Det'd.	Error	Recovery	Calc.	Det'd.	Error	Recovery
1. Wheat leaf extract								
2. 0.1 <i>M</i> sucrose, 0.033 <i>M</i> $\text{KH}_2\text{PO}_4$	1035	310 1100*	5.9		1035	320 1075*	3.7	
3. Equal parts No. 1 and No. 2	705 705	700 695	0.7 1.4	99.0 98.0	710 710	700 700	1.4 1.4	100.1 100.1

\* Difference between  $P_i$  and  $P_t$  in No. 2 = 2.2%.

#### *Phosphorus in Organic Combinations*

Organic phosphates can be distinguished from one another by the differing solubilities of their barium salts (12) and also by the lability of their phosphate

groups when undergoing acid hydrolysis (6). The latter method has been employed in this laboratory whenever it has been unnecessary to identify the compounds as to species, but rather as to type. The procedure using a final concentration of 1*N* hydrochloric acid to effect hydrolysis (6) has been followed, separating the fractions into  $P_7$ ,  $P_{60}$ , and  $P_{180}$ , the subscripts referring to the time of hydrolysis in minutes. Total phosphorus ( $P_t$ ) is estimated by the wet ashing procedure using 60% perchloric acid. All these procedures depend on the liberation of inorganic phosphate groups during hydrolysis and the amount liberated is estimated by the difference between the inorganic phosphorus content of the original extract and the amount present after hydrolysis. For a discussion of the identifiable constituents of the various fractions, the reader is referred to Heard (6).

#### *Seven Minute Phosphorus ( $P_7$ )*

Transfer an aliquot, usually 1 ml., of the extract containing about 500  $\mu$ gm. P to a large boiling tube of the type used in sugar determinations. Add 4 ml. of distilled water and 5 ml. of 2*N* hydrochloric acid. Place the tube covered with a glass bulb into a boiling water bath. After seven minutes, cool rapidly and bring to pH 4.0 by the addition of 11 ml. of 1*N* sodium acetate. Transfer the contents of the tube quantitatively to a 50 ml. volumetric flask and make to volume with distilled water. A blank of distilled water is committed to the same procedure. Centrifuge, and use 1 ml. for the determination of phosphorus. The increase in inorganic phosphorus, if any, over the value determined on the extract prior to hydrolysis is called seven minute hydrolyzable phosphate. Table IV shows the procedure adopted to test the validity of the method on a sample of glucose-1-phosphate, an ester that liberates its phosphoric acid group under these conditions of hydrolysis.

#### *Sixty Minute and 180 Min. Phosphorus ( $P_{60}$ and $P_{180}$ )*

The procedure is essentially the same as for the seven minute phosphorus determination, except that the tubes are retained in the boiling water bath for their respective times. Each tube in this case is fitted with an air condenser consisting of a long glass tube (length, 55 cm.; inside diameter, 6 mm.) attached to the tube by means of a rubber stopper. Heard (6) found difficulty in obtaining consistent results unless the tubes were sealed. However, the present author has not encountered this difficulty and consistent results have been obtained by this method. Recovery tests for inorganic phosphate are shown in Table III.

#### *Total Phosphorus ( $P_t$ )*

Total phosphorus is determined by the wet ashing usually of 1 to 2 ml. of the initial extract containing about 500  $\mu$ gm. P, with 5 ml. of 60% perchloric acid in a small Kjeldahl flask. The flask and contents are heated in a slanting position over a small flame in a fume cupboard until the solution becomes colorless. A blank with distilled water is made at the same time. After cooling, the contents of the flask are washed quantitatively into a 50 ml. volumetric flask with 40 ml. of 1*N* sodium acetate and made to volume.

One ml. is used for the determination of inorganic phosphorus. The difference between this value and the value for inorganic phosphate in the original extract is considered to be total phosphorus in organic combination and is signified by  $P_u$  ( $P_u = P_t - P_i$ ). Parts of the  $P_u$  fraction may be identified in the  $P_7$ ,  $P_{80}$ , and  $P_{180}$  fractions.

Neutralization of the perchloric acid with a 2*N* solution of sodium hydroxide invariably gave high blank values, even if the alkali was stored in a paraffin-waxed container. This was assumed to be due to a continual dissolution of silica from the glass vessel, since the absorption spectra of such blanks were similar to that of the silica complex shown in Fig. 2, and freshly prepared solutions of alkali do not show this characteristic absorption. Although accurate results could be obtained if a standard amount of the alkali was added to the blank and the unknown each time, the use of sodium hydroxide was discontinued owing to this interference, and replaced by 1*N* sodium acetate. It was often more convenient to use the stronger buffer solution described previously.

#### *Recovery Tests of Inorganic and Organic Phosphates on Plant Extracts*

A Klett-Summerson photoelectric colorimeter was used in the recovery tests on plant extracts. Known amounts of potassium dihydrogen phosphate, and glucose-1-phosphate were added to extracts of wheat leaves. The results are shown in Tables II, III, IV, and V.

TABLE III

RECOVERY OF INORGANIC PHOSPHATE IN THE 180-MIN. PROCEDURE ON WHEAT SEEDLING EXTRACTS

Test material	$P_i + P_{180}$			
	$\mu\text{gm./ml.}$		%	
	Calc.	Det'd.	Error	Recovery
1. Wheat seedling extract		50.0		
2. No. 1 + 10 $\mu\text{gm. P}$	60.0	58.33 60.0	2.8 0.0	83.33 100.0
3. Wheat seedling extract		45.3		
4. No. 3 + 20 $\mu\text{gm. P}$	65.3	66.0	1.2	103.5

At first inspection it may appear that some of the recoveries especially the recovery of glucose-1-phosphate from wheat leaf extract as shown in Table V are too low or too high. However, it must be remembered that the absolute error of a method is involved in the percentage recovery. If the substance to be recovered is small in proportion to the whole, then a small percentage error will be manifested as an abnormally large deviation in percentage recovery. Thus, percentage recovery cannot be used alone as a criterion of validity, but

TABLE IV

LIBERATION OF PHOSPHATE FROM GLUCOSE-1-PHOSPHATE IN THE 7-MIN., 180-MIN., AND TOTAL PHOSPHATE PROCEDURES

Procedure	Apparent P.	Recovery
	$\mu\text{gm./ml.}$	%
1. 7-min. dist. water blank	0.0 (set)	
2. 7-min. + G-1-P* (room temp.)	1.0	
3. 7-min. + G-1-P*	15.8	100.5
4. 180-min. + G-1-P*	15.6	99.4
5. Total phosphate + G-1-P (wet ashing)*	16.0	101.8

\* Glucose-1-phosphate solution equivalent to 15.7  $\mu\text{gm. per ml. P.}$ 

TABLE V

RECOVERY OF GLUCOSE-1-PHOSPHATE FROM WHEAT LEAF EXTRACT

Test material	$P_i$			$P_i + P_r$				$P_t$			
	$\mu\text{gm./ml.}$		%	$\mu\text{gm./ml.}$		%		$\mu\text{gm./ml.}$		%	
	Calc.	Det'd.	Error	Calc.	Det'd.	Error	Recovery	Calc.	Det'd.	Error	Recovery
1. Wheat leaf extract		355							355		
2. G-1-P solution					15.7						
3. Equal parts No. 1 and No. 2	177.5	175.0	1.5	185.3	183.5	1.0	75.0 (on calc.) 99.5 (on det'd.)	185.3	185.0	0.2	96.0 (on calc.) 128.0 (on det'd.)

must be considered together with the percentage error either between determinations or between the theoretical and the experimental. In Table V, percentage recovery has been based on both the theoretical and the experimental value.

The greatest percentage errors are to be found in the difference between the calculated and experimentally determined values for phosphate in the phosphate buffer (Table II). However this is offset by the considerably lower error between phosphate determined by the normal and the total phosphate procedures and, furthermore, by the good recovery obtained from the wheat leaf extracts. On the whole the absolute error of the method lies within 2% and the results shown in Tables II to V inclusive are indicative of the validity of the procedures.

#### Phosphate Fractions in Wheat Leaves

One of the best methods by which the validity of a procedure is tested is to prepare a balance sheet of the substance under determination. Accordingly, data of this nature are presented here.

Wheat leaves of the Khapli variety, three weeks old, were harvested and passed through a mechanical juicer to separate the major part of the cell wall material. The juice was then filtered through cheesecloth and centrifuged for a few minutes to remove cell debris and initial determinations were made of the inorganic and total phosphate content of the juice. To 100 ml. of extract, 1.5 ml. of sodium acetate (1*N*) - acetic acid buffer pH 4.0 was added to lower the pH from the initial value of pH 5.61 to pH 4.5, at which pH value the colloidal suspension is readily precipitated. The extract was separated into residue and supernatant by centrifuging for about 10 min. and inorganic and total phosphorus determinations were made on 90 ml. of the supernatant, and on the residue made to a total volume of 80 ml. with distilled water. A phosphate balance sheet has been prepared, calculated on the basis of absolute quantities present (Table VI) and this also has been diagrammatically represented in the form of a histogram in Fig. 7.

TABLE VI  
BALANCE SHEET OF PHOSPHATES IN WHEAT LEAF JUICE

Fraction of juice	$P_i$		$P_t$		$P_u(P_t - P_i)$	
	Mgm.	% of total	Mgm.	% of total	Mgm.	% of total
Whole juice (W)	46.5	100	34.25	73.5	12.25	26.5
Supernatant (S)	33.75	72.6	35.33	76.0	-1.58	0
Residue (R)	12.40	26.7	6.44	13.8	5.96	12.8
S + R	46.15		41.77		4.38	
S + R - W	-0.35		7.52*		-7.87*	

\* Increase of  $P_i$  fraction equivalent to decrease in  $P_u$  fraction.

Phosphorus in organic combination is designated as  $P_u$  calculated from the difference between  $P_t$  and  $P_i$ . Whole juice in this case contains 26.3%  $P_u$ . and analysis of the supernatant after separation indicates that no  $P_u$  is present in this fraction. Therefore it should all be found in the residue, but analysis shows that only half the amount present in the original juice can be recovered in the residue (12.8%). Since the  $P_i$  in the supernatant (72.6%) accounts for all of the  $P_i$  present in the original juice (73.6%), none of this fraction should be present in the residue, but this is not the case and  $P_i$  is found in the residue approximately equivalent (13.8%) to the amount of  $P_u$  apparently lost from the residue. This juice was not deproteinized initially and hence changes are to be expected in the various fractions. It appears that the changes occur in the residue after dispersion in water and they are associated with a gradual release of inorganic phosphate ( $P_i$ ) from the organic phosphates ( $P_u$ ) probably due to the action of a biological system. This experiment, of course, does not indicate that the  $P_u$  fraction is confined solely to the residue. Similar phosphorus cleavage processes may have been occurring in the supernatant during the extraction procedure and subsequent treatment. In fact,

if juice is deproteinized immediately after extraction, organic phosphate fractions are revealed in the supernatant as shown in extract No. 2, Fig. 7. However, apart from this incidental physiological interest the data bear witness to the precision of the method, which audits the balance sheet of phosphates within permissible experimental error.

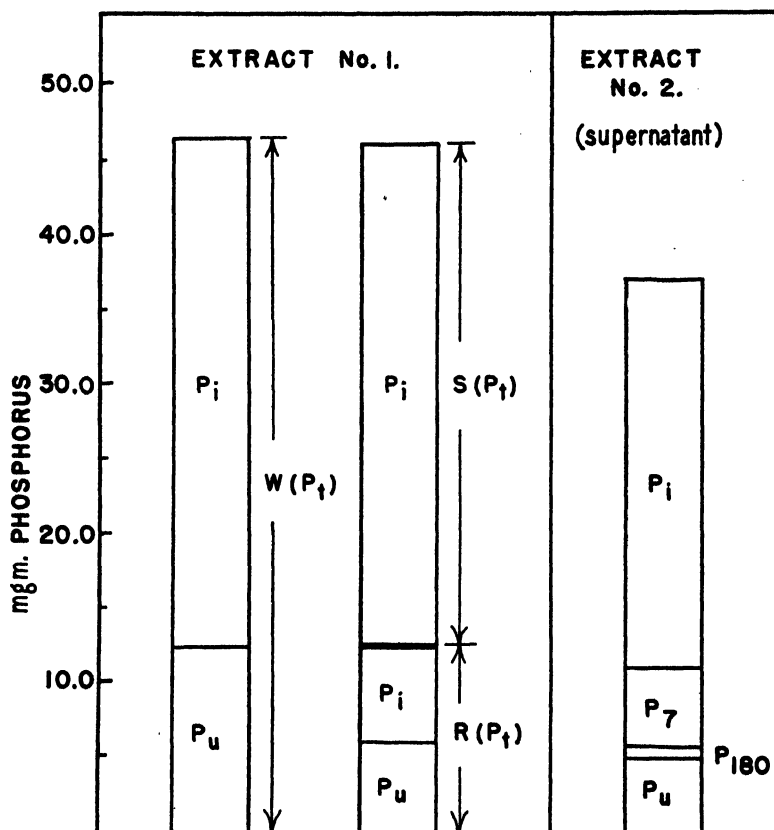


FIG. 7. Extract No. 1. Phosphate fractions in the whole juice, supernatant, and residue of wheat leaves; W = whole juice, S = supernatant, R = residue. Extract No. 2. A supernatant of wheat leaf juice revealing the presence of organic phosphate fractions.

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## STUDIES ON THE MICROFLORA OF FLOUR<sup>1</sup>

BY NORMAN JAMES<sup>2</sup> AND K. N. SMITH<sup>3</sup>

### Abstract

Bacteria and fungi, each cultured on appropriate media at 25° C., were found to constitute the major groups of organisms in five brands of commercial flour. Numbers of these organisms showed the same trend in relation to brands and the difference between brands was found to be significant. In addition, these brands harbored mesophilic acid-producing bacteria, thermophilic flat-sour spores, anaerobic thermophilic spores, spores of rope-producing bacteria, and yeasts. Numbers of organisms in the latter groups did not show a consistent relationship to brands. The usual method for determining spores of rope-producing bacteria proved to be inadequate for obtaining counts of spores of species known to produce rope in bread. Replication of sampling and careful laboratory procedures were found to be necessary for obtaining reliable estimates of any population in flour.

Wheat flour is the basic food for a large part of the world's population. Any factor that affects its quality is of economic importance to the farmer and the milling industry. Relatively little is known about the microflora of Canadian flour, although it might be expected that flour would harbor soil, dust, and grain types of bacteria, yeasts, and fungi that could affect the quality of products made from it. It would appear logical, therefore, that fundamental information on the flora of normal flour should be available as a background for studies involving specific defects that from time to time confront the milling and baking industries. This study was undertaken to provide some of this basic information.

### Historical

Tanner (9) reviewed the literature on the microbiology of flour. Early investigations established certain general facts. Flour harbored large and varying numbers of microorganisms, the difference in numbers being related to differences in moisture and in quality. Certain species of bacteria and of fungi were commonly encountered. Such defects as rancidity and mustiness in flour and ropiness in bread were associated with specific types of organism. Kent-Jones and Amos (8) outlined a procedure for obtaining counts of total bacteria and of spores of rope-producing bacteria in flour. These investigators reported that the washing of grain before milling caused a substantial reduction of bacteria in flour; that patent flours harbored relatively small numbers of bacteria; and that spores of rope-producing bacteria were present in most samples examined. Gustafson and Parfitt (4), using the technique of Kent-Jones and Amos, found that the development of rancidity during

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storage of flour was not related to changes in numbers of bacteria. Two years later Holtman (5), investigating the problem of rancidity, reported that over-bleaching tended to increase microbial oxidation of flour. Barton-Wright (2) attempted to find a relationship between bacteria and fungi and chemical changes during storage. He attributed mustiness of flour to fungi and not to bacteria. Of the fungi, 90% belonged to the genus *Penicillium*. Amos (1) found a species of *Streptococcus* and a torula in most samples of flour examined. He isolated from flour *Micrococcus ureae*, *M. liquefaciens*, and *Flavobacterium* sp. This investigator considered the last named to be epiphytic on wheat. James *et al.* (7), in an investigation on the microflora of stored wheat, reported that two types of bacterium, considered by them to be epiphytes, persisted on wheat even after repeated vigorous washings. They stated that the role of these bacteria in determining the properties of cereal products had not been established. Christensen (3) reported on a study on technique for the determination of molds in flour. This investigator found that molds were distributed uniformly in flour. A significantly higher count was obtained when a binocular microscope was used as an aid in counting numbers developing on plates.

### Preliminary Studies

The first study was designed to determine the effect of (a) time of shaking the initial dilution, (b) settling after shaking, and (c) difference in medium on counts of fungi in flour. A 10 gm. sample of flour diluted to 0.1 in sterile water and fine gravel was shaken for two minutes on a mechanical shaker. Immediately afterwards a 10 ml. aliquot was pipetted off to make a 0.01 dilution. The initial dilution was reshaken for 28 min., after which a second 10 ml. aliquot was transferred to make a second 0.01 dilution. The initial dilution then was permitted to settle for 10 min. before a 10 ml. aliquot of the supernatant liquid was used to make a third 0.01 dilution. Ten replicate plates were prepared from each of the three 0.01 dilutions. Czapek's agar, acidified by the addition of 1 ml. 10% lactic acid per 100 ml. of medium, was used in five plates; and malt-salt agar (3) in the remaining five. Incubation was at 25° C. for six days. The experiment was replicated 10 times, each with a different flour. The mean count per plate from 100 plates prepared from dilutions shaken for two minutes was 12.95; the mean count from the same dilutions shaken for 30 min. was 14.83; and the mean count from 100 plates prepared from the supernatant liquid was 2.73. The difference between any pair of the three means was found to be significant. The data were used, in addition, to compare counts on the two media. With Czapek's agar the mean count from 150 plates was 14.11, whereas with malt-salt agar it was 16.40. Again, the difference between means was found to be significant.

A second study was designed to determine the errors of sampling and diluting. Using aseptic technique and a sterile spatula about 100 gm. of flour were removed from a sack and mixed thoroughly by a standard procedure. Four replicate 10 gm. portions were diluted to 0.1 dilutions. These were shaken on a mechanical shaker for 30 min. Then from each, four 10 ml.

aliquots were used to make four replicate 0.01 dilutions. Each of these dilutions was shaken by hand 25 times. Then a 1 ml. aliquot was transferred to each of five plates for culturing in acidified Czapek's agar at 25° C. for six days. The experiment was replicated three times, each time from the same sack of flour. The experiment involved 240 plates, representing 48 dilutions, 12 portions, and three samples. The variation in counts between replicate samples was found to be significant; whereas the variation between replicate portions from one sample and the variation between replicate dilutions prepared from one portion were not significant.

### Microorganisms in Commercial Flour

Fifty samples, representing five brands milled by one company, were used. The brands were designated Nos. 1, 2, 3, 4, and 5, respectively, in ascending order of ash content. Duplicate samples of each brand, taken directly from the mill stream into sterile 6 oz. containers, were submitted at biweekly intervals, together with the results of routine analyses for ash, protein, and moisture on each brand at each interval. The bacteriological studies were carried out on the one-day-old samples. The system of mixing and diluting was based on the preliminary findings. Each initial dilution was shaken for 30 min. Each higher dilution was made before appreciable settling could take place. Brands 1 and 2 were raised to the 0.01 dilutions. Brands 3, 4, and 5 were further raised to the 0.001 dilutions. The medium for diluting was sterile water (plus gravel in the initial dilution), and not sterile sodium chloride solution, as used by Kent-Jones and Amos and by Christensen. There appears to be no practical reason for using sodium chloride. It is not used ordinarily for diluting water, milk, or soil for bacteriological counts. The final dilutions were used for preparing triplicate plates for each of:

- (a) Bacteria on nutrient agar at 25° C. for six days. Many investigators (4, 8) have used 37° C. for this determination. Since under normal conditions the bacterial flora of flour would not be exposed to 37° C., it appeared reasonable to use the lower temperature of incubation.
- (b) Bacteria on brom-cresol-purple dextrose tryptone agar at 25° C. for six days. This medium is used commonly for bacteria in canned products, sugar, and starch. In this study it was used primarily to obtain counts of acid-producers.
- (c) Fungi and yeasts on Czapek's medium at 25° C. for six days. This medium has been used widely in soil studies. Many species of fungi can be recognized readily on it.
- (d) Fungi on malt-salt agar (3) at 25° C. for six days. Counts on this medium were made in order to provide additional data for comparison with counts on Czapek's medium.

After transfers were made for the above plates each 0.01 dilution was heated at 90° C. for 30 min. to destroy vegetating organisms. Each heated dilution was shaken 25 times and used for each of:

- (e) Thermophilic flat-sour spores on brom-cresol-purple dextrose tryptone agar at 55° C. for 48 hr. One ml. from the heated 0.01 dilution was plated in each of 10 plates. A count of the acid-producers on the 10 plates multiplied by 10 represented the number of thermophilic flat-sour spores per gm. of flour.
- (f) Thermophilic spores on the plates for (e). In this case all the bacterial colonies were counted.
- (g) Anaerobic thermophilic spores in Bacto-liver broth at 55° C. for 72 hr. Two ml. from the heated 0.01 dilution were added to each of 10 tubes of medium. Acid production in one tube was accepted as evidence of the presence of at least one spore. A count of the tubes showing acid production multiplied by five represented the number of anaerobic thermophilic spores per gm. of flour.
- (h) Spores of rope bacteria in nutrient broth at 37° C. for 72 hr. (8). One ml. from the heated 0.01 dilution was added to each of 10 tubes of the medium. The presence of a characteristic pellicle was accepted as evidence of at least one spore. A count of the tubes showing pellicle formation multiplied by 10 represented the number of spores of rope bacteria per gm. of flour.

The chemical analyses and the results of this investigation are summarized in Tables I and II.

TABLE I  
AVERAGE\* ANALYSES OF THE FLOURS STUDIED, %

Ingredient	Brands				
	1	2	3	4	5
Ash	0.324 ±0.006	0.354 ±0.026	0.444 ±0.026	0.532 ±0.072	0.602 ±0.038
Protein	11.3 ±0.7	12.3 ±0.7	13.5 ±0.5	13.5 ±1.1	15.3 ±1.1
Moisture	13.6 ±0.3	14.0 ±0.4	13.7 ±0.1	13.9 ±0.3	13.9 ±0.4

\* Average from five determinations.

The raw data from plates prepared for bacteria on nutrient agar at 25° C. and for fungi on malt-salt agar at 25° C. were submitted to analysis of variance tests. These two groups represent counts that showed the same trend with brands. The results are shown in Tables III and IV.

Similarly the raw data from plates prepared for fungi on Czapek's and on malt-salt agar were submitted to an analysis of variance test. The results confirmed the finding in the preliminary experiment that the malt-salt agar produced significantly higher counts of fungi.

TABLE II  
AVERAGE\* NUMBER OF MICROORGANISMS PER GM. IN THE FLOURS STUDIED

	Brands				
	1	2	3	4	5
<i>a</i>	2750	7980	17,630	12,900	19,500
<i>b</i>	2010	6600	13,990	8700	16,600
<i>c</i>	39	555	526	1760	720
<i>d</i>	1150	1040	2260	2180	2850
<i>e</i>	1270	1140	3510	2630	3960
<i>f</i>	54	268	12	47	81
<i>g</i>	22	24	176	63	72
<i>h</i>	47	60	822	133	196
<i>i</i>	1	0	2	1	2
<i>j</i>	49	36	84	66	85

\* Average from 10 samples.

NOTE: *a* = Bacteria on nutrient agar at 25° C.

*b* = Bacteria on B.C.P. (brom-cresol-purple) dextrose tryptone agar at 25° C.

*c* = Acid-producers on B.C.P. dextrose tryptone agar at 25° C.

*d* = Fungi on Czapek's agar at 25° C.

*e* = Fungi on malt-salt agar at 25° C.

*f* = Yeasts on Czapek's agar at 25° C.

*g* = Thermophilic flat-sour spores at 55° C.

*h* = Thermophilic spores at 55° C.

*i* = Anaerobic thermophilic spores at 55° C.

*j* = Spores of rope bacteria at 37° C.

TABLE III  
SIGNIFICANCE OF VARIATION IN COUNTS OF BACTERIA ON NUTRIENT AGAR AT 25° C.

Source	D.f.	Mean square	F value	F at 5%
Dates	4	643.40	22.03	2.76
Brands	4	454.85	15.58	2.76
Dates × brands	16	168.44	5.77	
Error	25	29.20		

TABLE IV  
SIGNIFICANCE OF VARIATION IN COUNTS OF FUNGI ON MALT-SALT AGAR AT 25° C.

Source	D.f.	Mean square	F value	F at 5%
Dates	4	145.62	16.73	2.76
Brands	4	1632.77	187.67	2.76
Dates × brands	16	197.27	22.67	
Error	25	8.70		

## Discussion

The necessity for care at the various sampling steps in the procedure for determining numbers of organisms of any type in flour is obvious when it is considered that the count is made on a mere 0.01 or 0.001 gm. of the flour. This does not imply that emphasis should be placed on unnecessary refinements in technique or that the procedure need be complicated. In the preliminary study on sampling it was found that the variation in counts between replicate dilutions, or that between replicate portions of the mixed sample, was within the limits of experimental error for the method. The basic error of the procedure for obtaining counts by the plating technique had previously been established (6) as the variation between counts from replicate plates prepared from one dilution. This may be accepted as evidence that the procedure for mixing the sample and preparing dilutions was satisfactory. On the contrary, the finding of a significant difference between counts on replicate samples in the preliminary study and between samples of any brand tested on different dates points to the inadequacy of an estimate based on a single sample and to the need for replication of sampling.

Bacteria cultured at 25° C. and fungi cultured at the same temperature evidently constitute the major groups of organisms on freshly milled flours. These groups showed the same trend in relation to brands, and the difference between brands was found to be significant. This trend did not conform to expectancy on the basis of ash content. Brand 3 harbored more bacteria and fungi, cultured at 25° C., than did Brand 4, even though the ash content of Brand 4 was higher than that of Brand 3 on all samples of these brands tested. The protein content of Brand 3 was higher than that of Brand 4 in three of the five samples of each brand studied. The difference in protein content reflects a variation in the materials blended that conceivably might effect a difference in the microbial population of these brands. The data provide little evidence of the superiority of either medium in relation to the other for culturing bacteria at 25° C.; or of either of the media used for culturing fungi at 25° C. The brom-cresol-purple dextrose tryptone agar, even though yielding counts of bacteria about 25% lower, may be preferred because of providing specific information on numbers of acid-producers, in addition to that on total numbers. Similarly Czapek's agar for counts of fungi may be preferred because of its general use in certain studies on soil fungi. It should be recognized, however, that the populations developing on the two media used for bacteria are not identical and that the same holds true on the two media used for fungi. In this study counts of fungi on plates were made with the unaided eye. The use of a binocular microscope, magnification  $\times 10$  to  $\times 25$ , as prescribed by Christensen (3) for counting fungal growths, is open to question. Such magnification is not used in standard procedures for determining numbers of fungi in other products. Further, information is not available to indicate that growths not visible by the unaided eye represent slow growers merely. They may represent second generation growths from species that scatter spores. If so, they should not be counted.

As was found to be the case on wheat samples (7), yeasts in flour appear to vary erratically, with little evidence of consistency in numbers in relation to other types of microorganism, or to ash or protein content. Yeasts and acid-producing bacteria cultured at 25° C. conceivably could affect fermentation during the leavening process.

The finding of spores of thermophilic bacteria (cultured at 55° C.) in flour was not unexpected. They are present in soil and have been isolated from heating composts and manure. They are known to cause certain types of spoilage in canned products. In some cases their presence in the canned product has been attributed to the sugar used. They could withstand the cooking of the baked product and become active after it was removed from the oven and before it was cooled to the normal holding temperature.

The count of spores of rope-producing bacteria by the procedure used, at best, gives only presumptive evidence of the bacteria that could cause ropiness. It is merely a count of heat-resistant types that produce a characteristic pellicle on nutrient broth. Many species produce such a pellicle. In this study 78 pure culture isolates considered tentatively to represent rope bacteria because of pellicle formation were grouped on the basis of sugar fermentations. Of this number only 16 gave sugar reactions that agreed with those of *Bacillus mesentericus* or *Bacillus panis*, two species known to produce ropiness in bread. Of course, on the basis of probability a flour that contains a large number of such pellicle formers would be likely to contain the specific bacteria that produce ropiness. The problem of rope-producing bacteria in flour appears worthy of detailed study under controlled conditions.

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## NOTES ON SEED-BORNE FUNGI

### VI. SORDARIA<sup>1</sup>

BY R. F. CAIN<sup>2</sup> AND J. W. GROVES<sup>3</sup>

#### Abstract

In the examination of agricultural seeds for the presence of seed-borne fungi, five species of *Sordaria* have been isolated. Three species, *S. fimicola* (Rob.) Ces. & De Not. isolated from a wide variety of seeds, *S. humana* (Fuckel) Winter isolated from corn, and *S. setosa* Winter isolated from beets, are common coprophilous fungi. Two species, *S. inaequalis* Cain isolated from a variety of seeds, and *S. curvispora* Cain from carrot and celery seed are described for the first time. The fungi do not appear to be of any pathological significance.

#### The Genus *Sordaria*

The genus *Sordaria* was established by Cesati and De Notaris (4) for a miscellaneous assemblage of species of sphaeriaceous fungi. From the time of its origin it has been used by different authors to include various groups of species most of which have a coprophilous habitat. One of the largest of these groups has appendaged spores, that is spores with either primary or secondary appendages or both. The characters used to limit the genus have varied from time to time in the different treatments.

The genus as understood at present includes a number of groups of more or less unrelated species. Various attempts have been made to divide the genus, but most of these divisions have been based on single characters and have resulted in an artificial separation of species showing natural affinities into genera comprised of unrelated forms. For example, *Pleurage* was separated by Griffiths (6) largely on the basis of ascus dehiscence. The asci of many of the species included by him in the genus are perforate at the apex just as in the species included by the same author in *Sordaria*. Likewise, species with asci containing more than eight spores have been grouped in the genus *Philocopra*. This character does not delimit a natural group but has arisen in various lines of evolution in several groups.

The genus *Sordaria* is retained largely on the basis of usage by the present authors in the more comprehensive meaning to include species in which the spores are variously ornamented with a gelatinous sheath, primary appendage or secondary appendage, or various combinations of these. Used in this sense the genus includes various groups of species probably not closely related,

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but detailed knowledge of their morphology and life history is still too inadequate to make an acceptable partition into separate genera, although this will ultimately be advisable.

If it is found desirable to retain the name *Sordaria*, it should be added to the list of genera conservanda and a lectotype chosen. This choice should be made with considerable care in order to avoid future confusion. When first used by Cesati and De Notaris in 1863 (4) 16 species were included although four of these were not transferred from *Sphaeria*. The first of these, *S. sordaria* (Fr.) Ces. & De Not., although somewhat doubtful, is now generally included in the genus *Rosellinia* or a segregate of it. *S. fimeti* (Pers.) Ces. & De Not. is placed in *Hypocypa* by Fries. *S. coprophila* (Fr.) Ces. & De Not., unlike most of those with appendaged spores, belongs to a different group closely related to or included in the genus *Bombardia* Fries. This species should not be chosen as the lectotype of *Sordaria* as was done by Clements and Shear (5).

*Sordaria fimiseda* Ces. & De Not. is the only other well known species with appendaged spores included by Cesati and De Notaris. If *Sordaria* is to be conserved for a group with appendaged spores, this species should be selected as the lectotype. It should be pointed out, however, that this species is a synonym of *Podospora fimicola* Cesati, which is the type of the genus *Podospora* established by Cesati (3) in 1856 antedating *Sordaria*. To use *Podospora* in the more comprehensive sense will require the transferring to it of a number of species having nonappendaged spores. Furthermore the genus *Podospora* is a synonym of *Schizothecium* established by Corda in *Icones Fung.* 2:29. 1838.

*Sordaria fimicola* (Roberge) Ces. & De Not. (not *Podospora fimicola* Ces.) seems to be the more logical choice as a lectotype for *Sordaria*. In this species the spores have a gelatinous sheath instead of appendages. It is included in the group more frequently assigned to *Sordaria* when this genus is used in a more restricted sense.

A number of coprophilous species that are evidently not closely related to either *Sordaria fimicola* (Rob.) Ces. & De Not. or *Sordaria fimiseda* Ces. & De Not. have been included in the genus *Sordaria*. Two of these groups have been segregated by the senior author (2) and transferred to *Coniochaeta* and *Bombardia* respectively.

To recapitulate, the genus *Sordaria* should ultimately be divided into several genera that would include species with natural affinities. As a basis for this division, a more adequate knowledge of the morphology and development of the species is required. An attempt should be made to establish the status of the various generic names now available for these segregated groups.

Some of the species, such as *Sordaria fimicola* (Rob.) Ces. & De Not., which are usually found on dung, frequently occur on other substrata, such as decaying vegetation. It is, therefore, not surprising to find a few species belonging to this genus among a large number of seed samples. The five species that have been encountered belong in three distinct groups. The first



of these includes *S. fimicola* and *S. humana* the spores of which have gelatinous sheaths but no appendages. *S. setosa* belonging to the second group has a primary appendage as well as secondary appendages on the spore. In the third group are two species described as new that show a relationship with *Sordaria curvula* De Bary.

***Sordaria fimicola*** (Rob.) Ces. & De Not., Comm. Soc. Crit. Ital. 1 : 226. 1863. (Fig. 1)

The mycelium of this species produces a rapid and luxuriant growth on malt agar. Numerous pyriform perithecia are soon produced in a superficial layer. These are black when viewed with reflected light but dark brown with transmitted light. The outer wall is bare except for the hyphae that surround the basal part and attach the perithecium to the substratum. There is a single palisadelike layer of cylindrical, eight-spored asci that are attached at the base and surrounded by a layer of hyaline nutritive cells. The spores are obliquely uniseriate, dark brown to nearly black, ellipsoid, rounded at the ends but more acutely below with a circular germ pore at the lower end. A broad, hyaline gelatinous layer surrounds the spore but does not cover the germ pore at the base. The spores produced in cultures are much more variable in size than those grown on dung. In the cultures obtained from seeds they measure  $19-27 \times 11-15\mu$  but mostly  $23-24 \times 12-14\mu$ . This is slightly larger than those grown under natural conditions where they are much more uniform in the same perithecium and in different collections, measuring  $19-23 \times 11.0-12.5\mu$ . A somewhat similar abnormality has been noted in cultures obtained from dung.

*Sordaria fimicola* has been isolated from seeds of *Apium graveolens* L. var. *dulce* DC. (celery), *Beta vulgaris* L. (mangels), *B. vulgaris* L. var. *cicla* L. (Swiss chard), *Brassica oleracea* L. var. *botrytis* L. (cauliflower), *Capsicum annuum* L. (pepper), *Cucumis sativa* L. (cucumber), *Cucurbita maxima* Duch. (squash), *C. Pepo* L. (pumpkin), *Daucus carota* L. var. *sativa* DC. (carrot), *Festuca rubra* L. (red fescue), *Glycine max* Merr. (soybeans), *Linum usitatissimum* L. (flax), *Lycopersicon esculentum* Mill (tomato), *Pastinaca sativa* L. (parsnip), *Phaseolus vulgaris* L. (beans), *Pisum sativum* L. (peas), *Raphanus sativus* L. (radish), *Spinacia oleracea* L. (spinach), *Taraxacum kok-saghyz* Rod (Russian dandelion), *Trifolium hybridum* L. (alsike), *Triticum aestivum* L. (wheat), *Vicia Faba* L. (broad beans).

This species has been isolated from seeds from Prince Edward Island, Quebec, Ontario, Manitoba, and British Columbia in Canada, from Connecticut and Michigan in the United States, and from Holland and the U.S.S.R.

***Sordaria humana*** (Fuckel) Winter, Botan. Z. 30 : 835. 1872. (Fig. 2)

This species closely resembles *Sordaria fimicola* but can be distinguished by means of the spores, which are distinctly broader in proportion to their length. In the cultures from seeds these measure  $22-28 \times 16-19$  (mostly  $23-25 \times 16-18\mu$ ). As in the previous species, the spores are more variable in size when grown in culture compared with those produced under natural conditions.

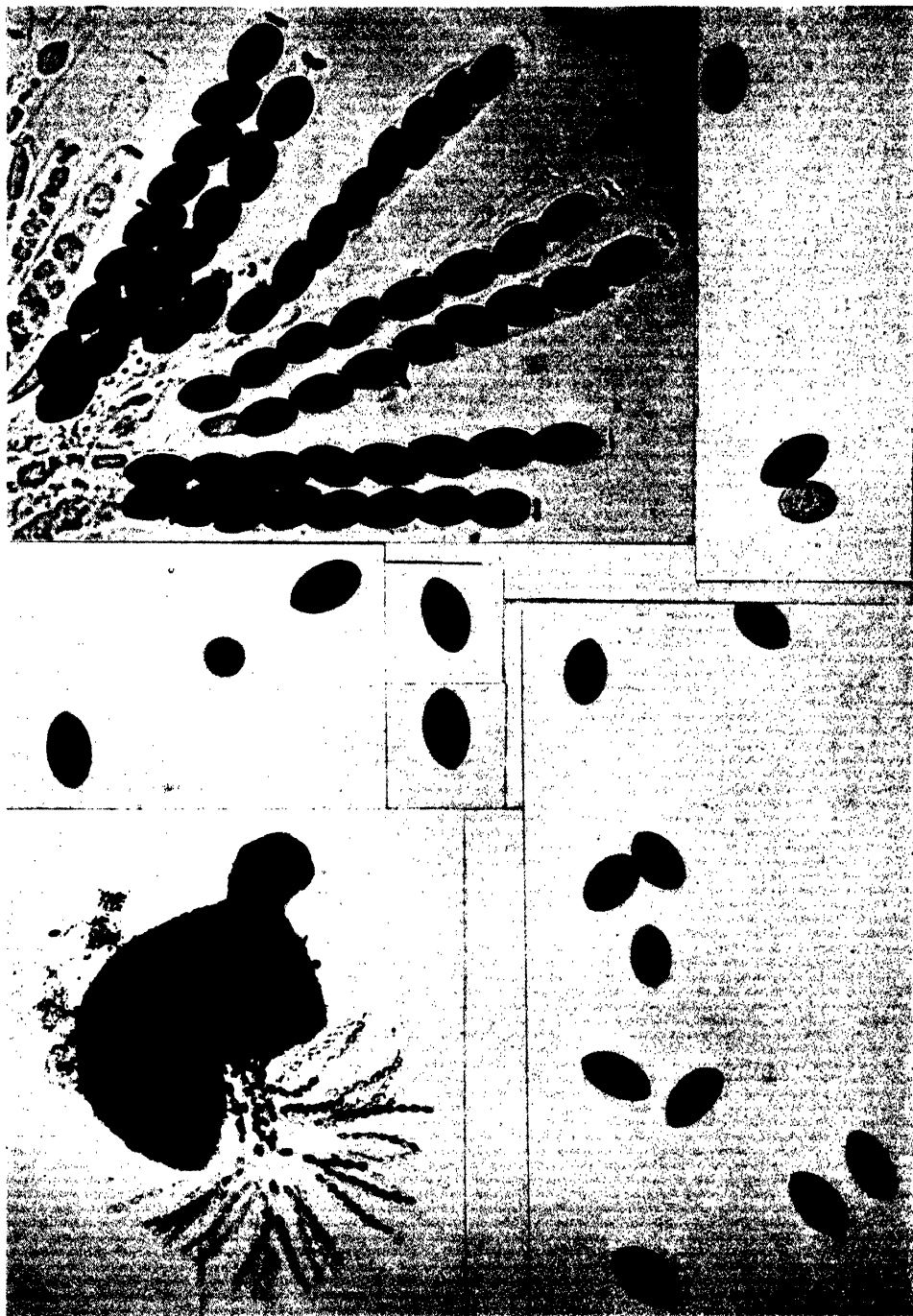


FIG. 1. *Sordaria fimicola*. Microphotographs of crushed perithecia, asci, and ascospores. Note the distinct pore in the apex of the ascus. The gelatinous sheath surrounding the ascospores is not evident.

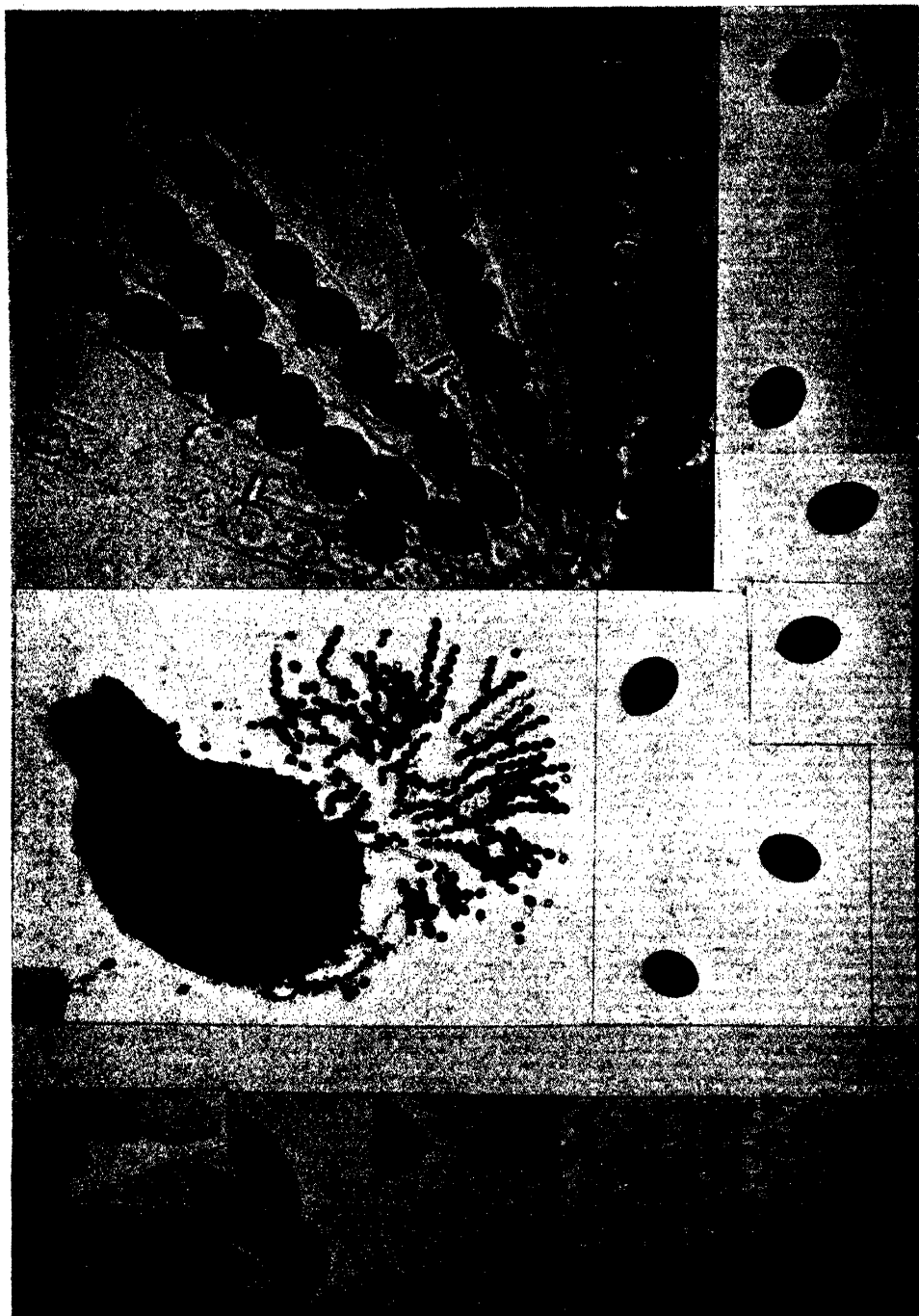


FIG. 2. *S. humana*. Microphotographs of crushed perithecium, asci, and ascospores.

FIG. 3. *S. inaequalis*. Microphotographs of ascospores. Note the hyaline, circular germ pore in the spore at the left.

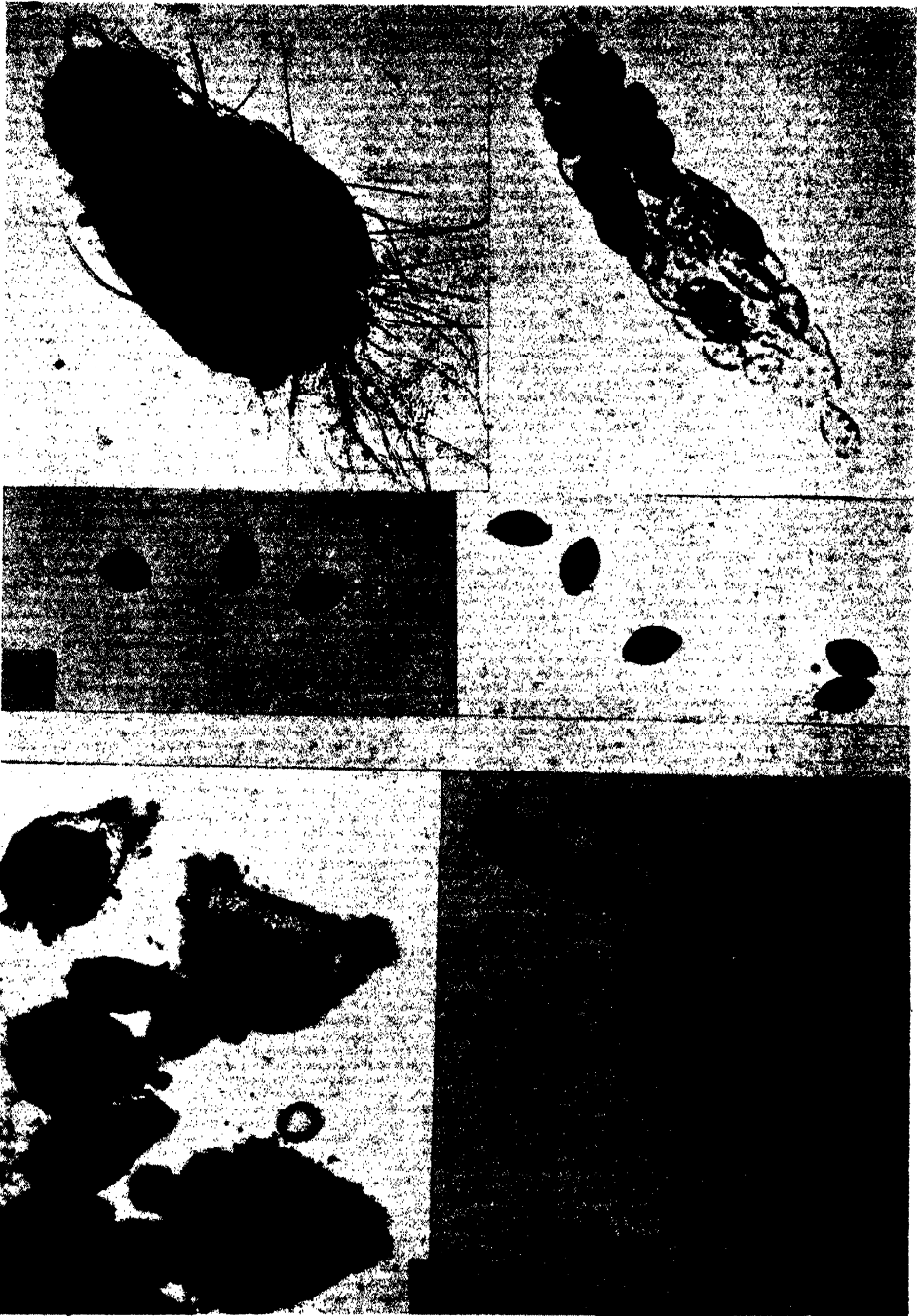


FIG. 4. *S. setosa*. Microphotographs of crushed perithecium, showing scattered hairs, ascus with numerous spores, and ascospores.

FIG. 5. *S. curvispora*. Microphotographs of perithecia (one showing two necks and two ostioles), ascus, and ascospores.

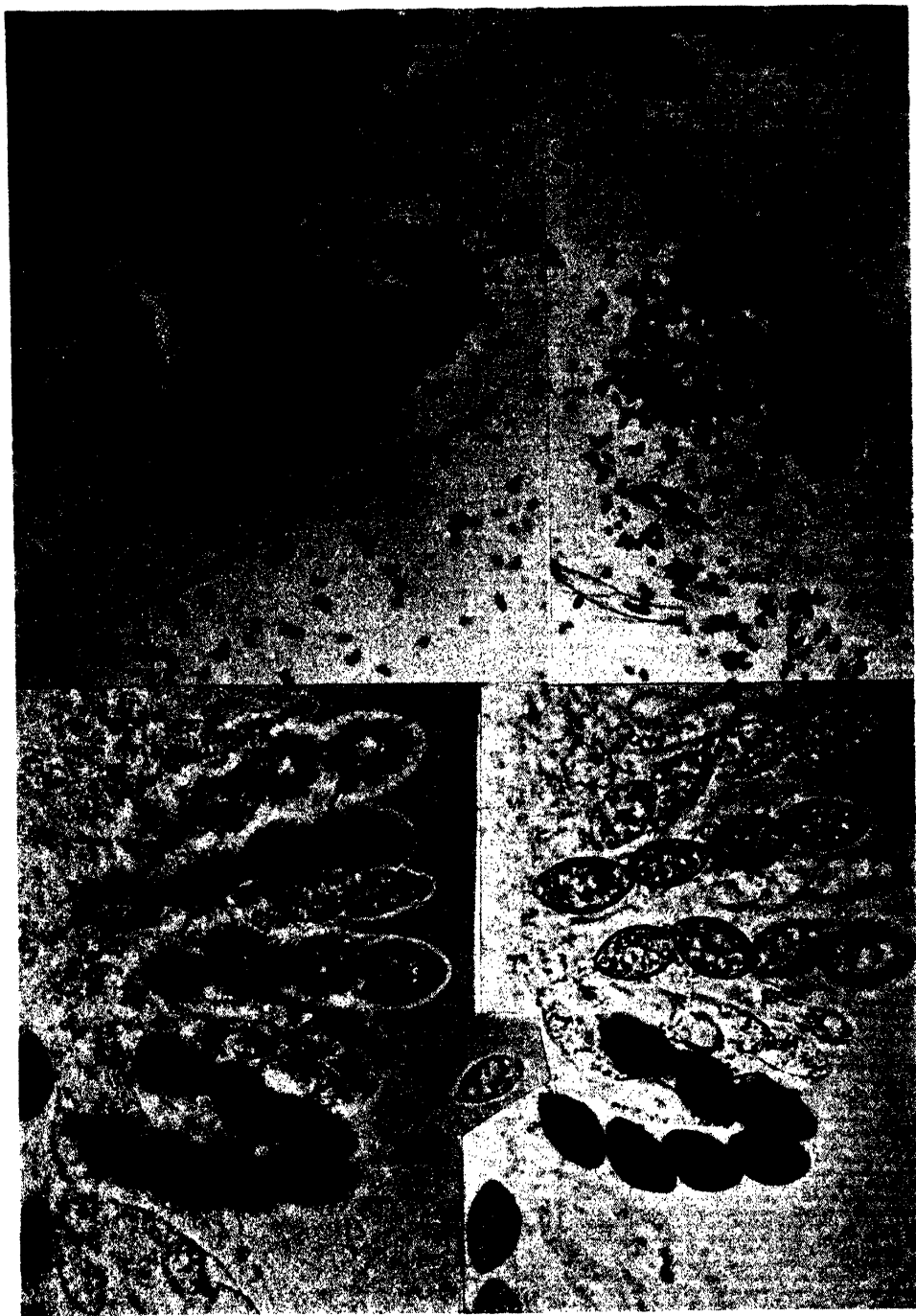


FIG. 6. *S. inaequalis*. Microphotographs of perithecia, with vesicular hair cells covering the outside, four-spored asci, and ascospores. Note the immature, hyaline spore by itself in the central lower half of the plate showing the primary appendage directed to the left.



FIG. 7. *S. curvispora*. Microphotographs of perithecia, asci, and ascospores. In the lower right is shown a highly magnified upper part of a perithecium showing the neck region with ostiole and the covering layer of fimbriate hair-cells. Note the germ pore in the more highly magnified ascospore near the center of the plate.



*Sordaria humana* has been isolated only once from seeds of *Zea Mays* L. (corn) from the Horticulture Division, Central Experimental Farm, Ottawa, Ont.

***Sordaria setosa*** Winter, Abh. Nat. Ges. Halle, 13 : 97. 1873. (Fig. 4)

Cultures of this species isolated from seeds produce numerous pyriform, olivaceous-brown perithecia. The papilliform necks are roughened with minute black papillae. The upper part of the perithecium and neck is covered with scattered, straight, faintly septate, brown hyaline-tipped hairs, which measure  $150-250 \times 3-4 \mu$ . The lower part has a dense covering of slender hyphae. The asci are fusiform-clavate and contain numerous spores. The ascospores are ellipsoid, dark brown and opaque, measuring  $17-21 \times 11-13 \mu$ . This size is somewhat smaller than that of the spores produced under natural conditions where they measure  $19-24 \times 11-16 \mu$ . Each spore has at the base a hyaline cell or primary appendage, which measures about  $11 \times 3 \mu$ . This cell and the apex of the spore each has a long slender lashlike secondary appendage.

*Sordaria setosa* has been isolated only once from seeds of *Beta vulgaris* L. (beets) from British Columbia.

***Sordaria inaequalis*** Cain sp. nov. (Figs. 3, 6, 8 to 15)

Peritheciis dense aggregatis, superficialibus, elongato-ovatis, elongato-conicis, interdum subcylindricis, parvis,  $240-430 \times 110-180 \mu$ , olivaceo-atris; peritheci membrana semitranslucida, tenui, e cellulis magnis, tenui tunicatis, transverse elongatis,  $10-25 \mu$ , constituta, cellululis ovatis, conglutinatis, semitranslucidis, olivaceis, tuberculatis,  $10-20 \mu$  diam., vestita; collo brevo, crasso, pilis elongatoribus vestito, periphysibus praedito. Ascis tetrasporis, cylindraceis,  $90-115 \times 11-14 \mu$ , superne late rotundatis, basi in stipitem  $15-30 \mu$  longum attenuatis. Sine paraphysibus sed cellulis magnis, hyalinis, vesiculiformibus praeditis. Ascosporis plerumque oblique monostichis, assymetricis, altera parte prope recta, altera valde fornicata,  $17-22 \times 10-13 \mu$ , initio hyalinis, dein olivaceo-nigris et opacis, basi appendice primaria, clavata, hyalina,  $4-8 \times 1-2.5 \mu$ , ornatis, appendicibus secundariis destitutis, foramine orbiculato, subapice, praeditis.

**Type:** In seminibus *Dauci carotae* L. var. *sativae* DC., Minneapolis, Minn., U.S.A., Feb. 8, 1940, TRT 22440.

Perithecia densely aggregated, superficial, elongate-ovate, elongate-conical, or nearly cylindrical, small,  $240-430 \times 110-180 \mu$ , olivaceous-black; wall semitransparent, thin, consisting of large thin-walled transversely-elongated cells measuring  $10-25 \mu$  in diameter, covered on most of the outside with a layer of ovate, nearly transparent, olivaceous cells measuring  $10-20 \mu$  in diameter that usually terminate in one or sometimes two short tubercles; toward the apex of the perithecium these become more slender as well as more densely and uniformly aggregated; neck short, broad, merging imperceptibly into body of perithecium, lined with periphyses. Asci four-spored, cylindrical,  $90-115 \times 11-14 \mu$ , broadly rounded above, tapering below into a stipe  $15-30 \mu$



long. No distinct paraphyses but with large, hyaline, vesicular, nutritive cells. Ascospores uniseriate, lying obliquely or sometimes parallel, inequilaterally ellipsoid, flattened to almost a straight line on one side,  $17-22 \times 10-13 \mu$ , hyaline when young, becoming olivaceous and then olivaceous-black and opaque; primary appendage clavate, hyaline,  $4-8 \times 1-2.5 \mu$  at base of spore; germ pore circular, on side with greatest curvature just below apex.

**Type:** Isolated in culture from seeds of *Daucus carota* L. var. *sativa* DC. Minneapolis, Minn., U.S.A. Crypt. Herb. Dept. of Botany, University of Toronto 22440. Additional specimens from same isolation in Herb. Div. Botany and Plant Pathology, Dept. of Agriculture, Ottawa. DAOM 15200 and in Herb. RFC 12049.

*Sordaria inaequalis* has been isolated from seeds of *Allium cepa* L. (onion), *Beta vulgaris* L. (beets), *B. vulgaris* var. *cicla* L. (Swiss chard), *Daucus carota* L. var. *sativa* DC. (carrot), *Pastinaca sativa* L. (parsnip), *Petroselinum hortense* Hoffm. (parsley), and *Pisum sativum* L. (peas).

It has been isolated from seeds from Ontario in Canada, and from Connecticut, Pennsylvania, Ohio, Illinois, Minnesota, and California in the United States.

Cultures of this species on malt agar produced rapidly growing, grayish colonies with abundant aerial hyphae forming a fluffy mass. Hyphae  $1.5-3.0 \mu$ , sparingly branched, remotely septate, very light brown, forming small strands.

The perithecial initial (Fig. 9) starts as a small inconspicuous coil on a short side branch (Fig. 8). No spermatia were seen.

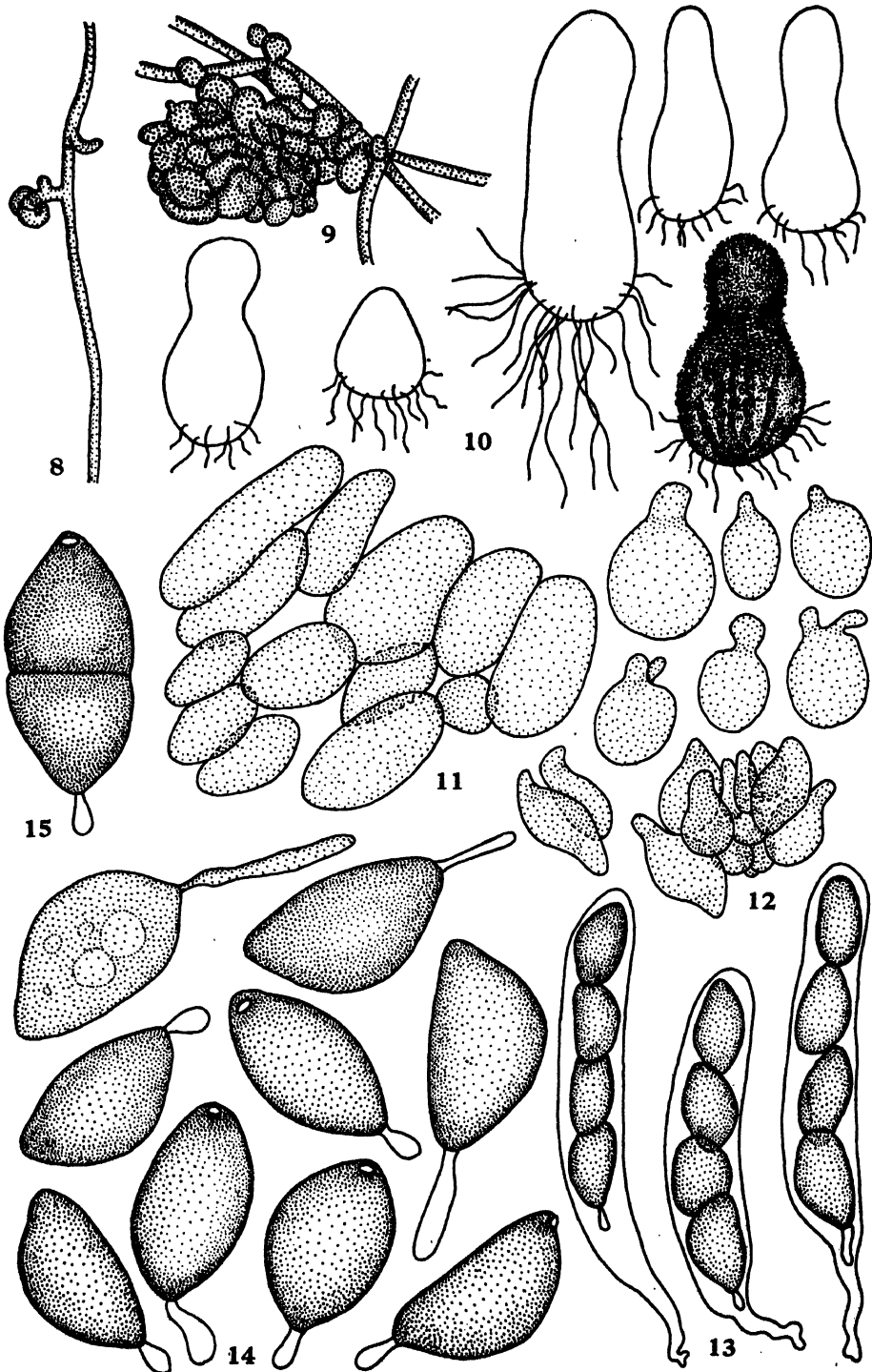
In some cultures many abnormal types of perithecia were produced as illustrated in Fig. 10. The normal shape is illustrated in Fig. 6. In one instance an abnormal spore with a transverse septum in the dark part was seen (Fig. 15).

This species represents a specialized development from a group of species of which *Sordaria curvula* De Bary is a typical representative. The group includes *S. tetraspora* Wint. (asci four-spored), *S. minuta* Fuckel (asci eight-spored, cylindrical), *S. curvula* De Bary (asci eight-spored, clavate), *S. coronifera* Grove (like *S. curvula* but larger throughout), *S. glutinans* Cain

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#### EXPLANATION OF FIGURES

FIGS 8 to 15. *S. inaequalis*. FIG. 8. Ascogonial coil as a side branch on a hypha. Note the small projection at the base of the coil that will participate in the formation of the wall of the perithecium.  $\times 960$ . FIG. 9. Primordium of perithecium showing the early development of the wall around the ascogonium.  $\times 960$ . FIG. 10. Outlines of perithecia showing various shapes, mostly somewhat abnormal. The more normal shape is shown in FIG. 6.  $\times 90$ . FIG. 11. Cells from outer layer of perithecial wall.  $\times 1300$ . FIG. 12. Hair cells that cover the perithecium. The lower ones are from the region around the ostiole, the upper from the mid portion of perithecium.  $\times 1300$ . FIG. 13. Three asci with mature spores.  $\times 600$ . FIG. 14. Ascospores showing the primary appendage and germ pore. The spore at the upper left is immature and nearly colorless with an abnormally long primary appendage.  $\times 1300$ . FIG. 15. An abnormal spore with a transverse septum.  $\times 1300$ .



FIGS. 8 to 15.

(with long, flexuous as well as agglutinated hairs), *S. dubia* Hansen (asci 16- to 32-spored), and *S. fimbriata* Bayer (asci eight-spored, spores inequilateral). *S. cervina* Cain and *S. linguiformis* Cain may belong in this series but have a different type of secondary appendage.

Of these species *Sordaria fimbriata* has characters that relate it more closely with *S. inaequalis*. The former has eight instead of four spores in the ascus. The spores are very similar except that they are somewhat larger in the four-spored species as would be expected if this were derived from the eight-spored species. In *S. inaequalis* the perithecia are smaller but otherwise similar, the agglutinated hairs are reduced in size and the end cells somewhat less fimbriate. It seems logical to conclude that this species has been derived from a form very similar to *S. fimbriata* by certain types of specialization.

The latter species, like *S. curvula*, produces spermatia in phialides. Several species having a similar type of spermatial production have been described in the genera *Phialophora* and *Cadophora*. Cultures of *S. inaequalis* have been searched to locate spermatia and phialides but, so far, none have been found. Probably the ascospores contain two nuclei when delimited in the ascus so that the cultures from monospores would be self fertile as in *S. anserina* (Ces.) Wint. The spermatia thus becoming functionless have apparently been lost.

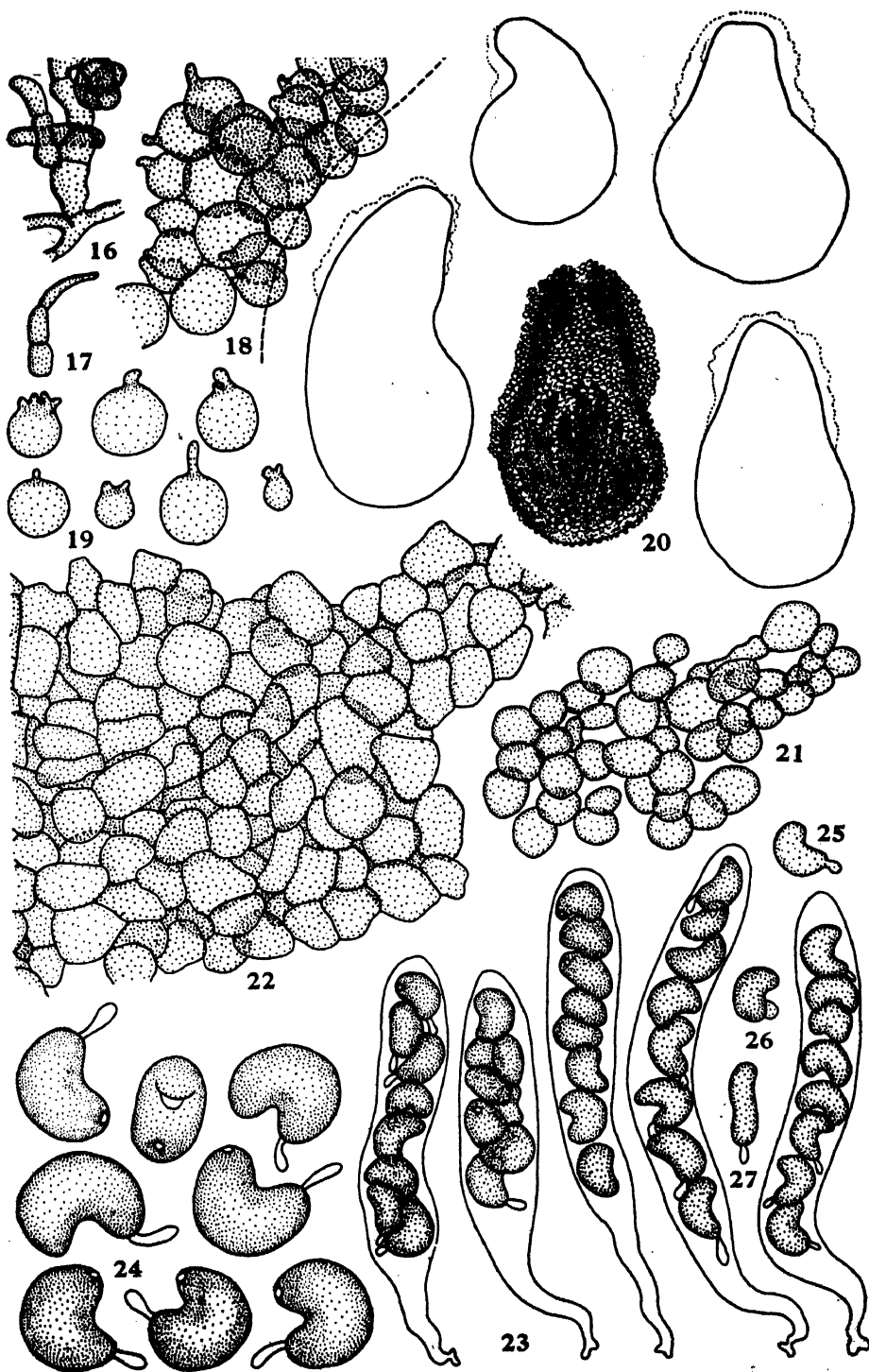
There is nothing in the description of *Bombardia lunata* Zickler (8) to indicate that it is specifically distinct from *Sordaria fimbriata* Bayer (1) so that it should probably be reduced to synonymy under the latter name, which has priority. It is possible that *Sordaria vratislaviensis* A. Schmidt (7, p. 32) is the same species and if so, is the proper name to use since it is older than either of the other two.

### ***Sordaria curvispora* Cain sp. nov. (Figs. 5, 7, 16 to 27)**

Peritheciis dense aggregatis, superficialibus, elongato-ovatis vel oblongo-conicis, 350-450  $\times$  200-230  $\mu$ , olivaceo-atris; peritheciis membrana tenui, semitranslucida, pallido-brunnea e cellulis magnis, ventricosis, usque ad 20  $\mu$  in diametro, constituta cellulis ovatis, usque ad 15  $\mu$  diam., conglutinatiss, pallido-brunneis, tuberculatis, vestita; collo brevo, crasso, pilis elongatioribus filiformibus, 0-2-septatis, dense aggregatis vestito, periphysibus praedito.

#### EXPLANATION OF FIGURES

FIGS 16 to 27. *S. curvispora*. FIG. 16. Ascogonial coil showing two branches from the basal cells that will participate in the development of the perithecial wall.  $\times$  960. FIG. 17. Single hair from the region of the ostiole.  $\times$  840. FIG. 18. Agglutinated hair cells forming the outside covering of perithecium taken from the base of the neck.  $\times$  840. FIG. 19. Terminal cells of hairs showing the tubercular projections at the apex.  $\times$  840. FIG. 20. Five perithecia.  $\times$  90. FIG. 21. Agglutinated hair cells covering the perithecium, taken from the mid region.  $\times$  840. FIG. 22. Cells from outer layer of perithecial wall.  $\times$  540. FIG. 23. Five asci with mature spores. The ascus that is second from the left has swollen out after standing in a water mount.  $\times$  840. FIG. 24. Ascospores showing primary appendage and germ pore.  $\times$  1300. FIG. 25. Ascospore in which a septum has failed to separate the primary appendage so that the entire cavity has become light brown.  $\times$  840. FIG. 26. Dark brown ascospore with a light brown appendage separated by a septum.  $\times$  840. FIG. 27. An abnormal ascospore that has failed to develop the normal curvature.  $\times$  840.



FIGS. 16 to 27.

*Ascis octosporis*, cylindraceis,  $100-110 \times 11-13 \mu$ , superne late rotundatis, apice obscure perforatis, basi in stipitem brevem attenuatis. Sine paraphysibus sed cellulis magnis, hyalinis, vesiculiformibus praeditis. Ascosporis incomposite monostichis, valde curvatis, altera parte valde impressa, altera valde fornicata,  $11-13 \times 6-8 \mu$  (interdum minus curvatis et tunc usque ad  $17 \mu$  longum), denique fuscis brunneo-atris et opacis; basi appendice primaria, clavata, hyalina, parva, circa  $5 \times 1 \mu$ , ornatis, appendicibus secundariis destitutis; apice foramine orbiculato praeditis.

**Type:** Ex seminibus *Dauci carotae* L. var. *sativae* DC., Gilroy, Calif., U.S.A., Feb. 8, 1940. TRT 22439.

Perithecia forming a dense layer on surface of agar, superficial, elongate-ovate to oblong-conical,  $350-450 \times 200-230 \mu$ , olivaceous-black, covered with a layer of hairs consisting of a few light-brown globose cells up to  $15 \mu$  in diameter, the terminal of which have one small projecting papilla (or occasionally up to three or four); hairs agglutinated and forming a dense layer, not developing in tufts, decreasing downward and disappearing near base of perithecium, gradually changing upward into the small, slender, filiform, tapering, 1-3-celled hairs that form a dense layer over the short, broad, papilliform neck; wall thin, semitransparent, light-brown, composed of rather large swollen cells that reach a diameter of  $20 \mu$ . Asci eight-spored, cylindrical,  $100-110 \times 11-13 \mu$ , broadly rounded above, indistinctly perforate at apex, tapering below into a short stipe, surrounded by a layer of hyaline ventricose cells but with no paraphyses intermixed. Ascospores irregularly uniseriate, strongly curved so that one side is concave,  $11-13 \times 6-8 \mu$  (sometimes less curved and then reaching a length of  $17 \mu$ ), becoming dark brownish-black and opaque, with a small circular apical germ pore; primary appendage basal, clavate, hyaline, small, measuring about  $5 \times 1 \mu$ . No secondary appendages.

**Type:** Isolated in culture from seeds of *Daucus carota* L. var. *sativa* DC., Gilroy, Calif., U.S.A. Crypt. Herb. Dept. of Botany, Univ. of Toronto 22439. Additional specimens from same isolation in Herb. Div. Botany and Plant Pathology, Dept. of Agr. Ottawa, DAOM 20504 and in Herb. RFC 12075.

*Sordaria curvispora* has been isolated from seeds of *Apium graveolens* L. var. *dulce* DC. (celery) and *Daucus carota* L. var. *sativa* DC. (carrot). Both isolates were from seed from California.

This species, like the preceding, seems to be related to *S. fimbriata*, being distinguished by having the spores smaller and more distinctly curved with a slightly smaller primary appendage. The terminal cells of the agglutinated hairs are less fimbriate.

Occasionally the primary appendage is not separated from the spore by a septum and in that case becomes brown.

The perithecial initial develops from a coiled ascogonium (Fig. 16) that is surrounded by irregular hyphae that appear to grow out from cells at the base of the ascogonial cell.

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## A DIAGRAMMATIC SCALE FOR ESTIMATING RUST INTENSITY ON LEAVES AND STEMS OF CEREALS<sup>1</sup>

BY R. F. PETERSON,<sup>2</sup> A. B. CAMPBELL,<sup>3</sup> AND A. E. HANNAH<sup>4</sup>

### Abstract

The Cobb scale and the modified Cobb (U.S.D.A.) scale were devised for estimating, by means of diagrams, the proportion of the area of a leaf or stem occupied by rust pustules. Existing diagrams illustrating these scales are inadequate in being arranged in intervals that are too large and irregular, and in not depicting a sufficient range of pustule sizes. New diagrams are presented using smaller regular intervals of rust intensity and a greater range of pustule sizes, thus providing a basis for more objective and accurate determinations.

### Introduction

Diagrammatic scales are necessary for measuring rust intensity on leaves and stems of cereals. Such scales have been devised and published; and two of these, known respectively as the Cobb scale and the modified Cobb scale, have been widely used. Existing diagrams illustrating these scales, however, show certain inadequacies. The new diagrams here presented represent an attempt at improvement.

The first diagrammatic rust scale was published by Cobb (2) in 1892, and is reproduced in the present paper in Fig. 1. A full quotation of Cobb's description of this scale follows.

The following figures illustrate the scale of rustiness devised and used for this report. The scale is in five parts, representing five degrees of rustiness. The gray spots are to represent the rust, and the oblongs on which they occur represent a portion of the wheat leaf, one half inches long. The lowest degree of rustiness is that where one per cent of the surface of the flag or sheaths is covered by rust. The next highest degree is where five per cent of the surface is covered with rust. The three other degrees are where ten, twenty, and fifty per cent of the surface is covered. I found this scale very easy of application. A dozen samples were gathered at random; these were then passed one at a time over the above scale of diagrams when it was very easy to see which number of the scale was nearest to each leaf in turn. The result was entered in the notes accordingly, the plus and minus signs being used to indicate a slightly greater or slightly less amount of rust than that indicated by the scale number. Thus, 20 + would mean an amount of rust which covered slightly more than twenty per cent of the surface, and 20 - an amount which covered slightly less than twenty per cent. I found this scale so convenient and so much more expressive and accurate than the inadequate use of terms like "very rusty", "quite rusty", "rotten with rust", &c., &c., that I have reproduced here my original figures, and hope to see them made use of by others. I believe that the numbers are more expressive than any words, but would call attention to the fact that the terms "very slightly", "slightly", "considerably", "very considerably", and "exceedingly" have been used in this report to correspond with the above five degrees of rustiness.

It should be noted that in Cobb's diagrams the percentages given refer to the actual percentage of the leaf area occupied by rust pustules, and that although the highest reading shown in his diagrams is 50%, Cobb did not

<sup>1</sup> Manuscript received May 19, 1948.

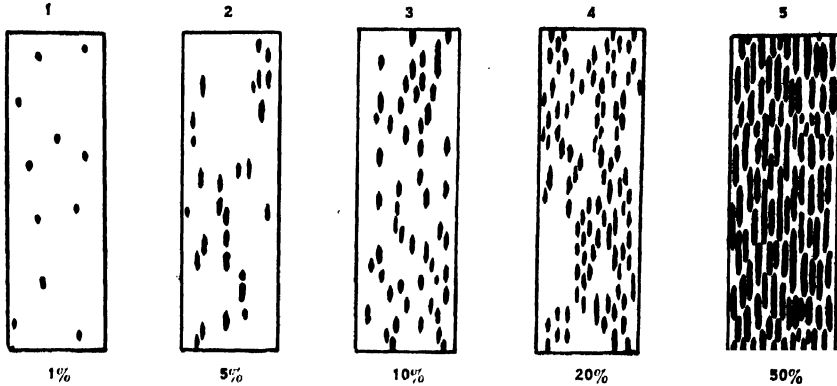
Published as Paper No. 147 of the Cereal Division, Experimental Farms Service, Dominion Department of Agriculture.

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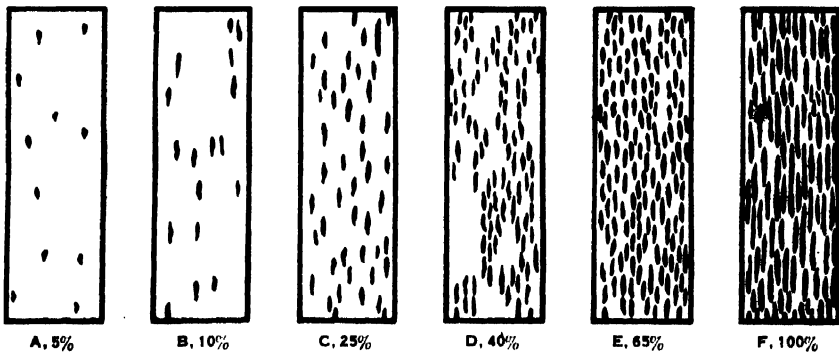
<sup>4</sup> Student Assistant, Dominion Laboratory of Cereal Breeding, Winnipeg.

# PLATE I



Five pieces cut from five different wheat leaves to show five different degrees of rustiness. The shaded spots represent rust.

FIG. 1. Scale for estimating rust and caption as published by N. A. Cobb. (Reproduced from the *Agricultural Gazette of New South Wales* by permission of the Editor).



Scale for estimating rust, illustrating six degrees of rustiness used in estimating the percentage of stem-rust infection. The shaded spots represent rust, and the figures represent approximately the rust percentages computed on the basis of the maximum of surfaces covered by rust as shown in the 100 per cent figure (F). Figure F in the diagram represents 37 per cent of actual rust-covered surface and is arbitrarily selected as 100 percent. The other percentages are in terms of Figure F.

FIG. 2. Scale for estimating rust and caption as published by Leo. E. Melchers and John H. Parker. (Reproduced from U.S. Dept. Agr. Bulletin No. 1046 by permission of the Chief of Publications, U.S. Dept. Agr.).



PLATE II

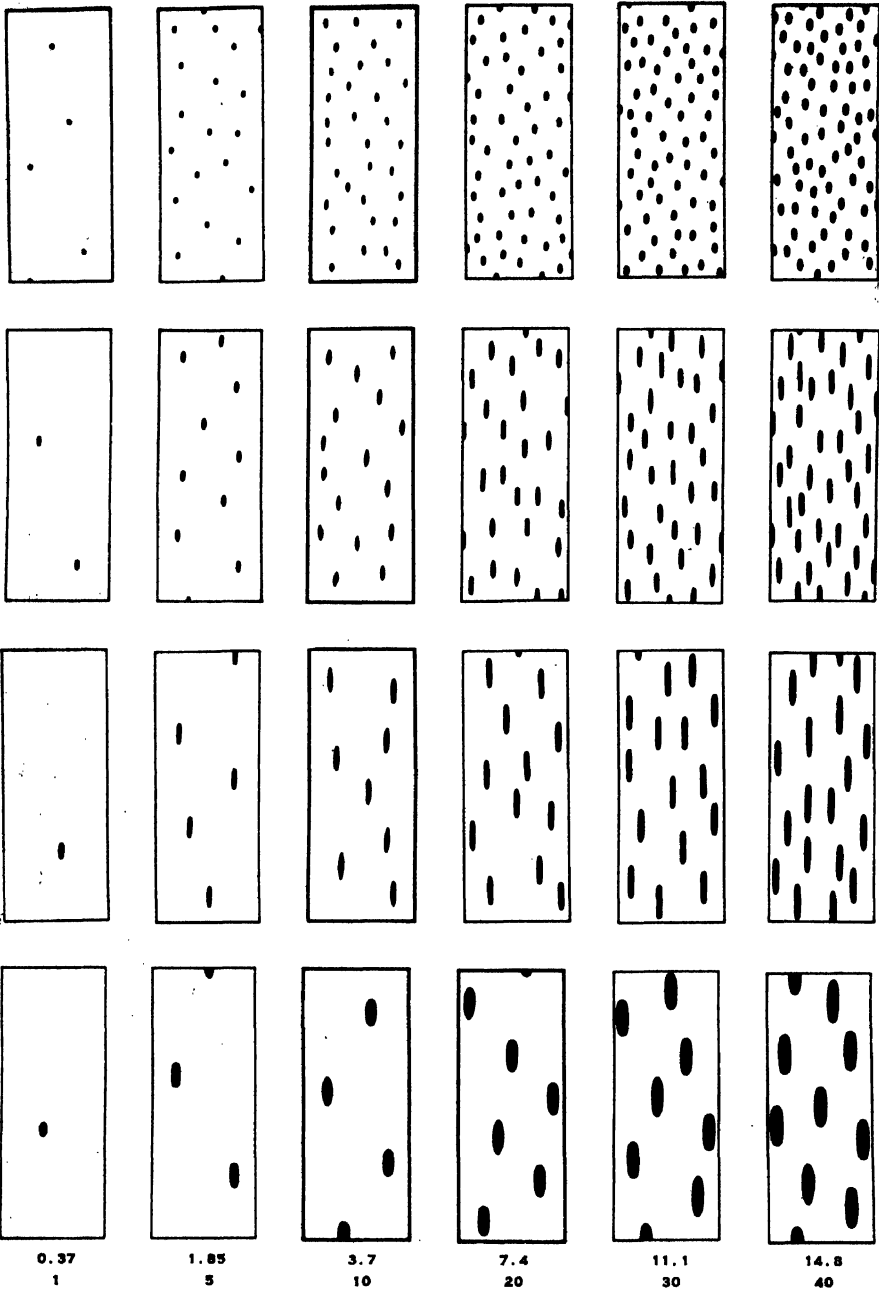


FIG. 3. Scale for estimating rust.

(A). Actual percentage of area occupied by rust pustules.

(B). Standard rust readings according to the U.S.D.A. scale. (See Fig. 2).

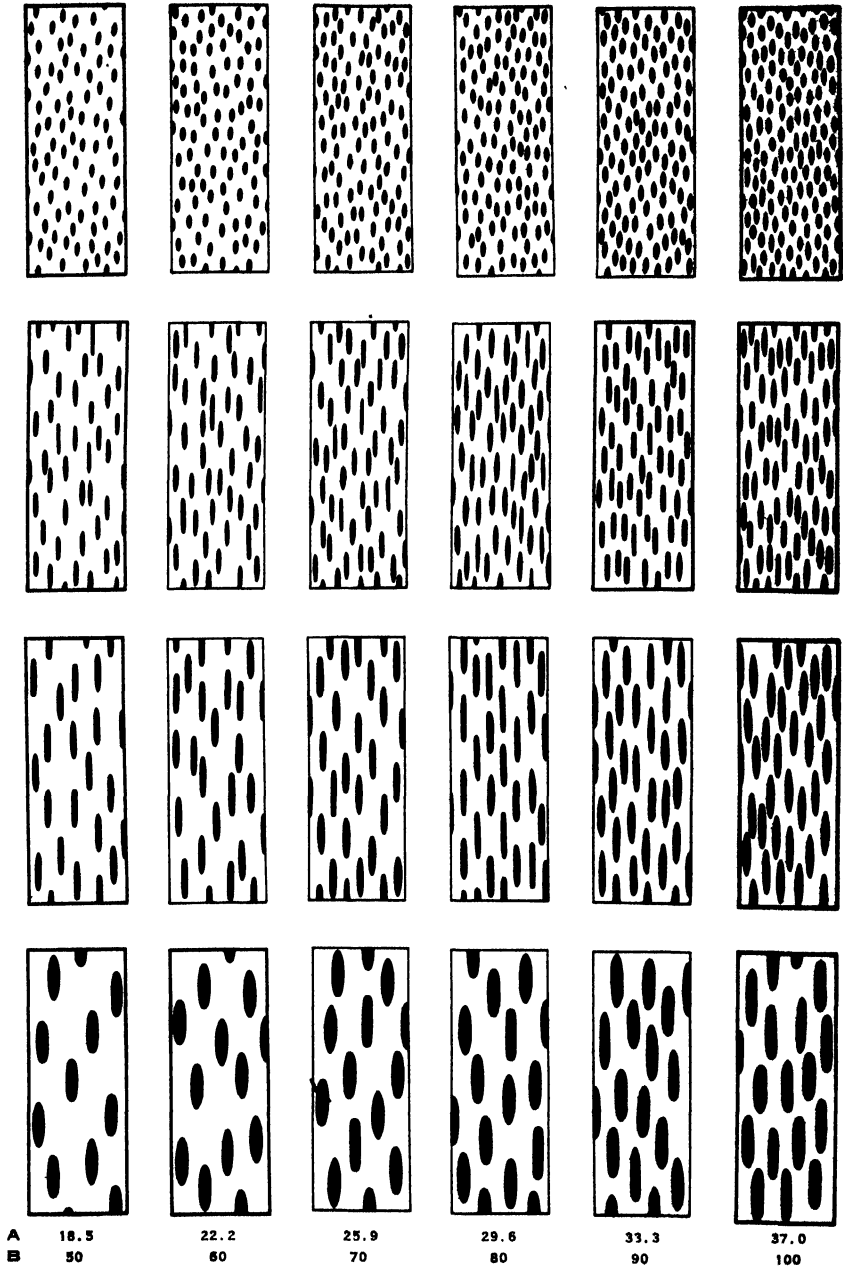


FIG. 4. Scale for estimating rust.

(A). Actual percentage of area occupied by rust pustules.

(B). Standard rust readings according to the U.S.D.A. scale. (See Fig. 2).

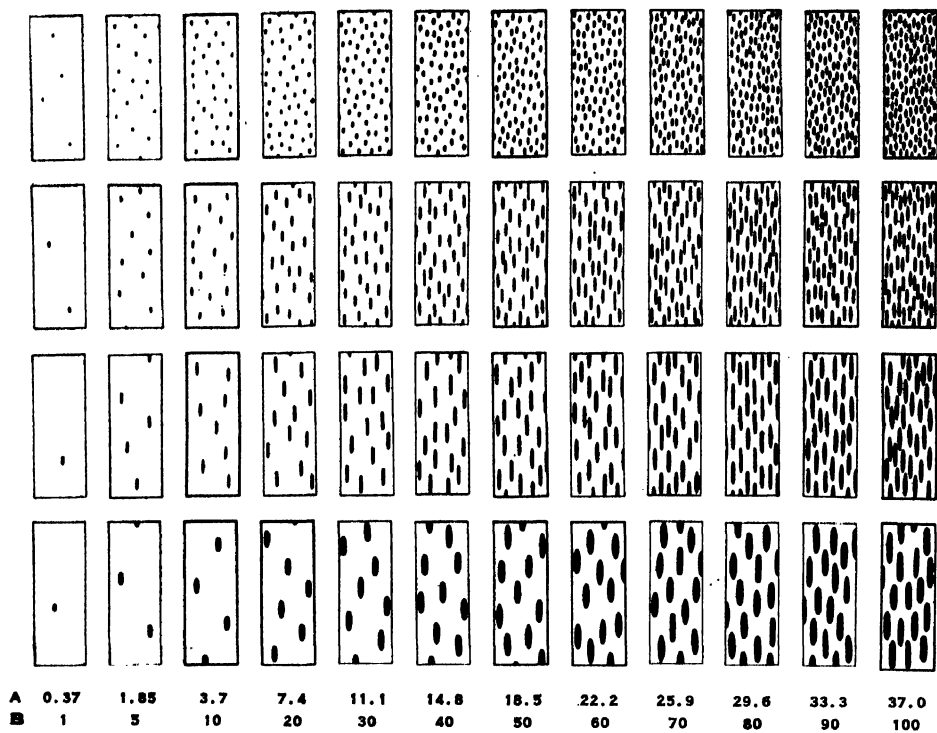


FIG. 5.

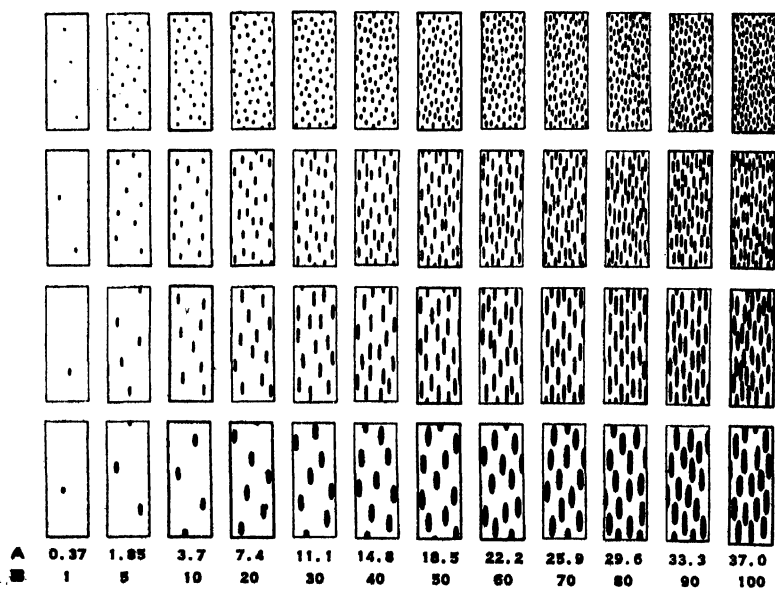


FIG. 6.

state or imply that this is a maximum reading. An investigator using Cobb's scale can, if he wishes, estimate rust percentages above 50%. The limit is obviously 100%, with the entire surface occupied by pustules. (This condition is sometimes attained on relatively small areas of leaves or stems of cereals where pustules have coalesced, but probably never on an entire leaf or stem). Cobb's scale then, if fully extended, provides for the estimation of the percentage of the area of a leaf or stem occupied by rust pustules through the full range from absence of rust to complete coverage of an area by rust pustules.

In 1917 the Office of Cereal Investigations of the Bureau of Plant Industry, United States Department of Agriculture, adopted a modification of the Cobb scale that was published by Melchers and Parker (4) in 1922. It is reproduced in the present paper in Fig. 2 and will be referred to here as the U.S.D.A. scale. In the development of this scale the diagram representing 37% of the total surface as occupied by rust pustules was arbitrarily selected as 100%, and the lower percentages were arranged in terms of this diagram. This procedure is based on the fact that mycelial development is more extensive than pustule development. The assumption is made that, in general, when pustules cover 37% of the surface, the development and destructiveness of the underlying mycelium are almost at a maximum. Supporting this assumption is the common experience that in the great majority of cases of cereal plants killed or badly damaged by rust, the total area of pustules on stems or leaves does not exceed 37% of the total area of those parts. The relatively few cases that exceed 37% are customarily classed as 100% when the U.S.D.A. scale is used.

The manner in which the originators of the U.S.D.A. scale derived their diagrams from those of the Cobb scale will be apparent from an examination of Figs. 1 and 2. Cobb's diagram having 50% of the total area shaded was apparently modified by removing enough of the shaded areas to leave 37% of the total area shaded. This was arbitrarily selected as 100%. The remaining diagrams were modified in a similar manner in order to have all percentages in terms of the arbitrary 100%. A new diagram (65%) was added.

Rust investigators have used the U.S.D.A. scale not only for classifying rusted plants in the six classes shown in Fig. 2, but also for estimating other percentages. Thus, in the bulletin by Melchers and Parker (4) the rust reactions of varieties are recorded by units from zero to five, and from there to 100 mainly in intervals of five, although smaller intervals are used in some cases. It appears that most other workers who have used the U.S.D.A.

#### EXPLANATION OF FIGURES

FIG. 5. Scale for estimating rust. Reduced reproduction of Plates II and III.

(A). Actual percentage of area occupied by rust pustules.

(B). Standard rust readings according to the U.S.D.A. scale. (See Fig. 2).

FIG. 6. Scale for estimating rust. Reduced reproduction of Plates II and III.

(A). Actual percentage of area occupied by rust pustules.

(B). Standard rust readings according to the U.S.D.A. scale. (See Fig. 2).

scale have recorded rust percentages in intervals of either 5 or 10, but sometimes in intervals of one, particularly in the low range from zero to five.

The U.S.D.A. scale has become the most commonly used basis for recording rust intensity on cereals in North America and is widely used in various countries throughout the world. The diagrams (Fig. 2) have been a great help to rust investigators but are inadequate for present-day use (as are also the original diagrams of Cobb) in the following respects:

1. Too few diagrams are shown, making a great deal of interpolation necessary when recording rust readings in intervals of 5 or 10%.
2. The irregular intervals used make classification difficult.
3. The depicted pustules do not adequately represent the great range in size of uredia and telia of rusts occurring on cereals.

The new diagrams here presented (Plates II, III, and IV) have been developed in an attempt to overcome, to some extent, the inadequacies of the older diagrams. They are intended for use in connection with uredia and telia of the following rust species:

*Puccinia graminis* Pers.

*P. rubigo-vera* (DC.) Wint.

*P. hordei* Otth.

*P. coronata* Cda.

The writers do not consider these diagrams suitable for stripe rust caused by *P. glumarum* (Schm.) Eriks. and Henn.

### The New Diagrams

Large scale diagrams (19.3 × 7 in.) representing portions of leaves or stems of cereal plants were drawn on white showcard; and areas representing rust pustules were outlined in pencil and filled in with India ink. A planimeter was used to measure the areas of the shaded portions of the diagrams. To represent 100% of rust on the basis of the U.S.D.A. scale the total area of pustules was arranged to be 37% of the area of the diagram, and lower percentages in the remaining diagrams were arranged in proper proportion. With the size of diagram used, 1% on the basis of the U.S.D.A. scale (0.37% of the total area) is 0.5 sq. in., a convenient unit for planimeter readings.

Instead of a single series of five or six diagrams as shown in Figs. 1 and 2, four series of diagrams with different pustule sizes were prepared, each series having 12 diagrams. After all pustule areas had been checked with the planimeter, individual photographs were made of the 48 diagrams and these were mounted in proper order in four series to be photographed again and reproduced as a single figure. The resulting scale is shown in Plates II and III. In order to provide a still greater range of pustule sizes this scale is reproduced in two different reductions in Figs. 5 and 6. Other reductions or magnifications of this scale can readily be made if this should be found desirable.

Both the actual percentages corresponding to the Cobb scale and the readings according to the U.S.D.A. scale are shown, so that the present scale is a dual purpose one. It was not possible to make this scale equally convenient (reading in multiples of 5 or 10) for both types of readings. It has been arranged to be most convenient for the readings corresponding to the U.S.D.A. scale, because this is at present the more commonly used of the two scales. The four horizontal series of diagrams are arranged so that the four diagrams making up any column have equal total areas of pustules.

The method of using the new diagrams in Plates II, III, and IV is to select a diagram in which the pustules most closely resemble, both in size and in density of spacing, the pustules on the leaf or stem under study. The reading given below the selected diagram will be the estimate of the proportion of the area of the leaf or stem that is occupied by pustules. A more objective and accurate reading is obtained in this way than by using only diagrams depicting pustules of average size. This point will be readily appreciated if the reader will examine the four diagrams in a given column (say the last column in Plate II) and the corresponding eight diagrams in Plate IV. Although each of these 12 diagrams has 14.8% of its area shaded, the visual impression of the proportion of area shaded varies from one diagram to another owing to changes in pustule size.

One of the difficulties encountered in using the older scales has been the classifying of leaves carrying extremely small pustules of *P. rubigo-vera*, *P. hordei*, or *P. coronata*. The writers have found the upper row of diagrams of Figs. 5 and 6 useful for cases of this kind.

Pustules differing markedly in size are sometimes found on the same leaf or stem. It has not been attempted here to represent pustules of varying size within individual diagrams because of the large number of possible combinations of pustule sizes. In actual practice it is often found that pustules of a certain size predominate in a given area of leaf or stem, and this facilitates the use of diagrams of the type provided.

### Discussion

It is not proposed to describe here the various published diagrammatic rust scales, nor to discuss the factors other than rust intensity (e.g., uredial infection type and stage of crop development) that must be considered in estimating crop loss due to rust. Chester (1) has recently published a discussion and bibliography dealing with these points, and Dickson (3) has illustrated the uredial infection types. The scope of the present paper is limited to the estimation of the proportion of the area of a given section of leaf or stem occupied by rust pustules.

A given reading in terms of the U.S.D.A. scale may be converted into an actual percentage reading by multiplying by the factor 0.37. Similarly, a given reading in actual percentage may be converted into a reading in terms of the U.S.D.A. scale by multiplying by the factor 2.7027. These relationships are true only in those cases where the area occupied by pustules does not

exceed 37% of the total area under consideration. Higher rust intensities than this are not distinguished by the U.S.D.A. scale, since it is customary to class them all as 100% rather than use numbers over 100.


Even if increases in rust intensity beyond the 37% point may have little significance in resultant destructiveness, most investigators would wish to be in a position to take such increases into consideration in critical studies. Thus the Cobb scale has an undoubted advantage over the U.S.D.A. scale in being more widely applicable in scientific work.

In most papers recording rust readings based on the U.S.D.A. scale the readings are below 100 so that the complication described above does not arise. In some papers, however, readings of 100 appear, and one is not certain whether the actual percentage is 37 or more. If investigators using the U.S.D.A. or similar scales would record estimates of the actual percentages represented by their relatively few recorded scores of 100, this complication could be avoided in all cases. The last diagram in Cobb's scale (Fig. 1) will aid in making such estimates.

### Acknowledgment

The writers are indebted to Mr. W. E. Clark, Technician, Dominion Laboratory of Plant Pathology, Winnipeg, for photographing all diagrams shown in Plates I to IV inclusive.

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The susceptibility of the above cultures, with the exception of *A. scabies*, to the antagonistic actinomycetes was studied by the flood plate technique. Suspensions of each organism were prepared in physiological saline from 4- to 6-day agar slants to a turbidity roughly equivalent to that of a 24-hr. broth culture of *E. coli*, and each suspension in turn was used to seed a small flask of cooled medium similar to that used for cultivation at a level of 1 to 1½% depending upon relative turbidity. In the case of *R. japonicum* and *C. sepedonicum* the agar was seeded at a level of 2 to 2½% to compensate for a somewhat slower growth. When the seed agar was prepared, 3.0 ml. was used to flood the surface of each asparagine agar plate having five actinomycete colonies of approximately five days' development. All plates were then returned to 26° C. for further incubation with the exception of those flooded with *E. coli*, which were incubated at 37° C. Cultures of *A. scabies* were tested by the cross streak method using a spore suspension in Tween "20" 1 : 5000 and a 3 mm. loop for cross inoculation of the original streak of selected antibiotic actinomycetes.

Antagonism of the test organism was estimated by measuring the diameter of the zone of inhibition after four days' incubation at 26° C. (with *E. coli* readings were taken after 24 hr. at 37° C.). Irregularities in colony shape as well as small differences in actual diameter of original spot inoculated actinomycetes were sources of error; however, the mean diameter measured to nearest millimeter was taken, since differences between duplicate plates prepared in early trials indicated that the uniformity to be expected allowed for measurement to this tolerance. With *A. scabies* the average of measured distance from growth to growth of streaks was taken as equivalent to the zone of inhibition.

### Comparative Antibiotic Effects

The antibiotic action of the 50 strains of actinomycetes on the 12 bacterial species (14 strains) is summarized in Table I. For convenience in presentation the degree of antagonism is indicated by the numbers 1 to 4, depending upon the extent of the zone of inhibition. This 'profile' determination indicates considerable differences in the bacterial susceptibility to the antagonistic organisms. Thus in the case of *C. sepedonicum* inhibitions were mostly at the maximum degree, whereas with most other organisms, there was a wider band of less pronounced response. The similarity of reaction observed with duplicate strains of *C. sepedonicum* and *A. scabies* testifies to the reliability of the method, zone diameters and intensity ratings following like patterns. Whereas the two species of *Azotobacter* showed very similar reactions to the antagonists, the three species of *Rhizobium* showed considerable variation in their susceptibility, particularly as regards intensity ratings.

It will be noted that only 78% of the antagonistic actinomycetes gave measurable zones of inhibition against *E. coli*, though they were selected originally because of antagonism exhibited against this organism. Whether



TABLE I  
PROFILE SUMMARY OF 50 ANTIBIOTIC ACTINOMYCETES

Test organism	Actinomycetes inhibiting		Average zone diam.	Degree of antagonism*				
	No.	%		4	3	2	1	0
<i>E. coli</i> (117)	39	78	21.1	16	4	13	6	11
<i>A. vinelandii</i> (534)	36	72	27.1	21	9	3	3	14
<i>A. chroococcum</i> (536)	36	72	25.2	19	8	5	4	14
<i>R. meliloti</i> (309)	38	76	19.2	8	10	16	4	12
<i>R. japonicum</i> (462)	30	60	26.6	19	5	3	3	20
<i>R. trifolii</i> (311)	40	80	23.2	20	4	12	4	10
<i>B. globiforme</i> (425)	47	94	25.1	21	9	14	3	3
<i>E. carotovora</i> (529)	38	76	21.4	11	10	14	3	12
<i>X. campestris</i> (589)	33	66	25.1	18	4	8	3	17
<i>X. phaseoli</i> (593)	36	72	23.7	13	10	11	2	14
<i>C. sepedonicum</i> (610)	47	94	29.7	32	9	6	0	3
<i>C. sepedonicum</i> (611)	48	96	30.2	35	6	7	0	2
<i>A. scabies</i> (597)	44	88	13.7	1	12	16	15	6
<i>A. scabies</i> (598)	44	88	14.0	1	12	16	15	6

\* NOTE: 4 = zone 30 mm. or greater.

3 = " 20 to 29 mm.

2 = " 10 to 19 mm.

1 = " trace to 9 mm.

0 = no inhibition.

this is to be ascribed to loss of the active strain in purification, mutation on cultivation, or decrease in intensity of antibiotic production within the limits of the test is not known. However, the latter explanation is in most cases most likely, since other species, e.g. *C. sepedonicum* and *B. globiforme*, were inhibited by 96 and 94% respectively of the actinomycetes.

The relative susceptibilities of the 12 test organisms to the 50 selected actinomycetes are shown in Fig. 1, where *E. coli* is taken as the standard reference with a rating of 100 and all other test cultures compared to this culture. Thus *C. sepedonicum* would appear to be the most susceptible organism tested, with *B. globiforme* and *A. scabies* following in decreasing order. This finding may be related to the fact that both *C. sepedonicum* and *A. scabies* are Gram-positive, while *B. globiforme*, in the characteristic coccoid stage, which it rapidly assumes on transfer, is also predominantly Gram-positive. It is not to be inferred that the degree of inhibition follows the same order as that shown in Fig. 1, for from Table I we may observe that *R. japonicum* when antagonized showed relatively large zones, yet this organism was acted upon by the least number of actinomycetes.

A further point of interest brought out by the survey is the relationship of the intensity of antibiotic effect exerted on the test organism to the numbers of test organisms acted upon by the actinomycetes. From Fig. 2 it is seen that those actinomycetes having the most intense antibiotic activity are, in general, the most versatile and act upon the greatest number of bacterial species.

The use of a single culture medium for the demonstration of antagonism is not without limitations, for antibiotic production is known to be regulated by many factors in the nutriment supplied. Since the antagonisms were

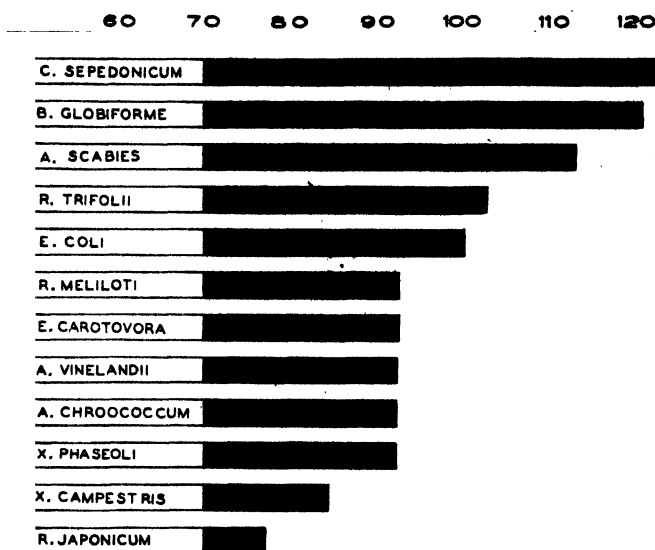


FIG. 1. Relative susceptibility of test organisms to antagonistic actinomycetes (*E. coli* = 100).

evident on a simple synthetic medium even greater effects might be expected under more favorable and varied conditions. Results based on a study of

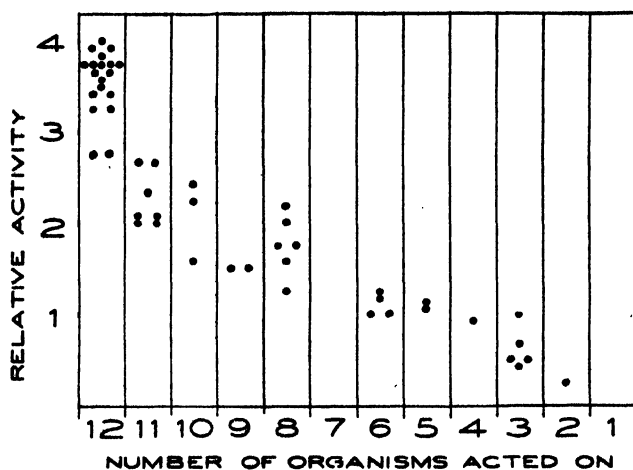


FIG. 2. Number of test organisms acted upon by 50 actinomycetes in relation to intensity of antibiotic effect.

the relationship of two isolated cultures are not necessarily applicable to soil with its heterogeneous microflora and vast possibilities of greater interactions. Though organisms possessing antagonist properties against a wide variety

of species may prove important in the establishment of bacterial balances in soil, yet those with greatest specificity may play an important role in the possible suppression of definite types, such as plant pathogenic soil-borne organisms.

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# GROWTH STUDIES OF NORMAL AND PARTHENOCARPIC TOMATO FRUITS<sup>1</sup>

By K. A. CLENDENNING<sup>2</sup>

## Abstract

Gaseous exchange by growing tomato fruits is localized in a ring of lenticel tissue that encircles the receptacle and is covered by the calyx. The size of this annular lenticel varies with the size of the fruit.

Growth of the fruits of the variety Grand Rapids includes a phase of residual mitotic activity that persists for approximately one week after setting. Cell division is reduced in dwarf or laggard fruits, which are examples of natural parthenocarpy. Under seasonal conditions that are favorable for natural setting, application of 0.2% indolebutyric - 50 p.p.m.  $\beta$ -naphthoxyacetic acid to pollinated flowers markedly increases the number and size of fruits obtained with the Marglobe but not with the Grand Rapids variety. The locular pulp of parthenocarpic fruits usually remains green during vine ripening. The green color is largely imparted by chloroplasts that are concentrated in the vicinity of the vascular strands leading to the aborted ovules.

## Introduction

As a result of numerous investigations, it is now well established that the exposed skin of tomato fruits is devoid of lenticels and stomata at all stages of development (7, pp. 560, 567). The available evidence also indicates that gaseous exchange by mature fruits in storage occurs chiefly in the stem scar region: blocking the stem scar area with wax results in a drastic inhibition of respiration and ripening (2, 3); a slight inhibition of ripening is also effected by leaving the stems attached to the fruit (2, 16). Less is known concerning the region of gaseous exchange in tomato fruits as they grow and ripen on the plant. Measurements of carbon dioxide emission *in situ* (by the mature fruit, calyx, and pedicel up to the abscission layer) revealed a pronounced respiratory climacteric (4). When a ring of wax was applied around the base of the stems, attached fruits did not ripen normally (4).

Information on the developmental stage at which cell division ceases in the flesh of the tomato fruit should improve our understanding of its normal and parthenocarpic growth. If mitosis continues for even a few days after anthesis, the effect of growth chemicals on cell division warrants consideration. Information on mitosis in the young fruit also assists in the interpretation of physiological data relating to successive stages of growth. For the stages in which there are no cell divisions, calculations that are made on a unit fruit basis refer to a constant cell population, whereas data expressed on a weight basis refer to widely different numbers of cells.

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, and the Department of Horticulture, Ontario Agricultural College, Guelph, Ont. Part of this paper is taken from a thesis submitted to the Graduate School of the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Issued as N.R.C. No. 1844.

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Agreement has not been reached as to the stage of development at which mitosis actually ceases. Smith (14) reported that cell division proceeds actively in the fruit flesh for approximately two weeks after pollination. Houghtaling (8) as well as Gustafson and Houghtaling (6) concluded that growth after pollination was caused entirely by cell enlargement. MacArthur and Butler (10) reported that ovary growth was entirely by cell division prior to pollination, and that subsequent growth was chiefly by cell expansion, cell division being a minor factor that just sufficed to maintain the epidermis, the cells of which do not expand. Groth (5) had previously reported that young and mature fruits contain the same number of epidermal cells, and that mitosis plays little part in the enlargement of the tomato fruit skin.

The yield and quality of fruits that are obtained following growth chemical applications to intact tomato flowers has been investigated extensively, notably by Howlett and Marth (9). From these and earlier studies, it has been established that growth and maturation can be accelerated, seed production curtailed, and the yield of marketable fruits increased under seasonal conditions unfavorable for natural setting (12). The large increases in yield reported by Howlett and Marth (9) were obtained with early spring populations of unspecified varieties. The interacting effects of growth chemical applications, varieties, and seasonal or environmental factors apparently have not been investigated.

This paper reports observations on the area of unrestricted gaseous exchange in the growing tomato fruit, and on the persistence of mitosis in the fruit flesh after anthesis and setting. The effects of spraying hand pollinated and emasculated flowers of the Grand Rapids and Marglobe varieties with 0.2% indolebutyric - 50 p.p.m.  $\beta$ -naphthoxyacetic acid solution have also been tested under conditions that were judged favorable for natural setting.

### Materials and Methods

The greenhouse populations employed in this investigation bore ripe fruit in July. The plants were propagated in soil essentially as directed by Walford (15). Daily tapping of the wire supports was used to effect self-pollination. The flowers were tagged at 'setting', or on the first day that wilting of the corolla was evident. Growth of individual fruits was followed by caliper measurements of the mean equatorial diameter. The number of cell layers composing the pericarp at successive stages of growth was counted radially at the equator. The observations summarized in Table II were made on stained microtome sections from a minimum of six ovaries or fruits. The counts were taken between vascular bundles at four different positions on each section. The cell counts on artificially induced and naturally parthenocarpic fruits were taken in the same manner on razor sections.

The treated and control fruits used in the waxing experiments occupied adjoining, randomized positions on the trusses, and were of similar size and age (diameter 30 to 45 mm., age 18 to 24 days). The sepals were removed

with forceps before the waxes were applied. Calyx removal was found experimentally to have no noticeable effect on subsequent growth and ripening. Hot paraffin (175° F.), and unheated vaseline and lanolin were applied as  $\frac{1}{4}$ -in. rings around the base of the pedicel.

The Grand Rapids and Marglobe plants (24 of each), employed in the growth chemical experiment, were grown simultaneously in adjoining benches. The treated and control flowers occupied adjoining, randomized positions on the third and fourth trusses. The growth chemical solution was essentially that which was found most effective by Howlett and Marth (9), namely 0.2% indolebutyric acid - 50 p.p.m.  $\beta$ -naphthoxyacetic acid. Carbowax 1500 (5%) was employed as a carrier. The individual flowers were sprayed once, with a nasal atomizer, and were shielded to prevent contamination of the adjacent controls. Maximum self-pollination of the 200 flowers referred to in Table III was effected by shaking and dusting them individually on the day that pollen was being freely shed. This treatment preceded spraying of the exposed flower parts. Flowers also were emasculated and sprayed when half-opened, or approximately two days before pollination would have begun. The growth chemical solution in this instance was sprayed directly on the pistil.

## Results and Discussion

### *Effects of Waxing the Region of Calyx Insertion*

All of the wax treatments tended to reduce subsequent growth and to inhibit ripening on the plant (Table I). Growth was stopped completely in two fruits treated with vaseline and in three of the fruits receiving paraffin, but

TABLE I

GROWTH OF TOMATO FRUITS FOLLOWING APPLICATIONS OF WAX TO THE CALYX REGION

(All fruits were waxed  $21 \pm 3$  days after setting at an initial diameter of 30 to 45 mm.)

Treatment	Number of fruits	Average increase in diameter, mm.	
		After two weeks	After four weeks
Control	24	11.9	16.7
Vaseline	12	7.7	9.7
Paraffin	12	6.0	8.5
Lanolin	12	3.9	6.8

complete blocking of subsequent growth was not observed with lanolin. Fruits that showed no subsequent growth assumed a leaden color within one week and finally shrivelled.

The most striking accompaniment of waxing was the appearance of large bubbles, usually within an hour or two, which on bursting provided a large breathing pore through the wax layer (Fig. 1). This phenomenon always occurred when lanolin was used, and the bubbles frequently rose to a height

of  $\frac{1}{2}$  to 1 in. above the wax collar before bursting. Repeated plugging of the resulting apertures over a two week period did not prevent fresh holes from appearing within a day or two. Because of this persistent breaking of the seal, the reduction of growth obtained with lanolin must have resulted from partial or intermittent blocking of the region of calyx insertion.

When clear mineral oil was used as the barrier on attached and detached fruits, it was possible to mark the point of bubble emergence with a needle. With detached fruits, bubbles were observed to rise from the ring of vascular bundles within the stem scar zone. On attached fruits, the bubbles always appeared in a ring of uncuticularized tissue that surrounds the receptacle and ordinarily is covered by the calyx (Fig. 2). This tissue forms a massive annular lenticel. Since gas bubbles did not emerge from the sepal scars at the base of the pedicel, the calyx apparently does not function in the respiratory exchange of growing fruits. Longitudinal sections through the lenticel tissue at the point of bubble emergence revealed vascular strands in close proximity. These strands lead from the outer fruit wall to the pedicel, and pass just beneath the annular lenticel.

Examination of mature fruits from different varieties revealed that lenticel ring width varies directly with fruit size. This relationship to fruit size was also evident in Grand Rapids fruits examined with an eyepiece micrometer at successive stages of growth:

Fruit diameter, mm.	10	20	25	35	50	65
Lenticel ring width, mm.	Visible	0.1	0.25	0.5	1.3	1.7

#### *Cell Division in the Pericarp After Setting*

Table II provides a summary of our observations on the number of cell layers comprising the pericarp of Grand Rapids ovaries and fruits at successive stages of growth. Cell division continued in the fruit wall after pollination and setting but ceased within the first week. No increase in cell number was

TABLE II  
NUMBER OF CELL LAYERS IN THE PERICARP OF GROWING TOMATO FRUITS

Stage of development	Ovary diameter, mm.	Pericarp cell layers
Flowers opening (1 day before pollination)	1.8	9
Flowers 'setting' (2 days after pollination)	3.0	14
After setting		
2 days	6.0	18 - 20
8 to 10 days	20.0	22 - 24
15 to 20 days	35.0	22 - 24
30 to 35 days	50.0	22 - 24

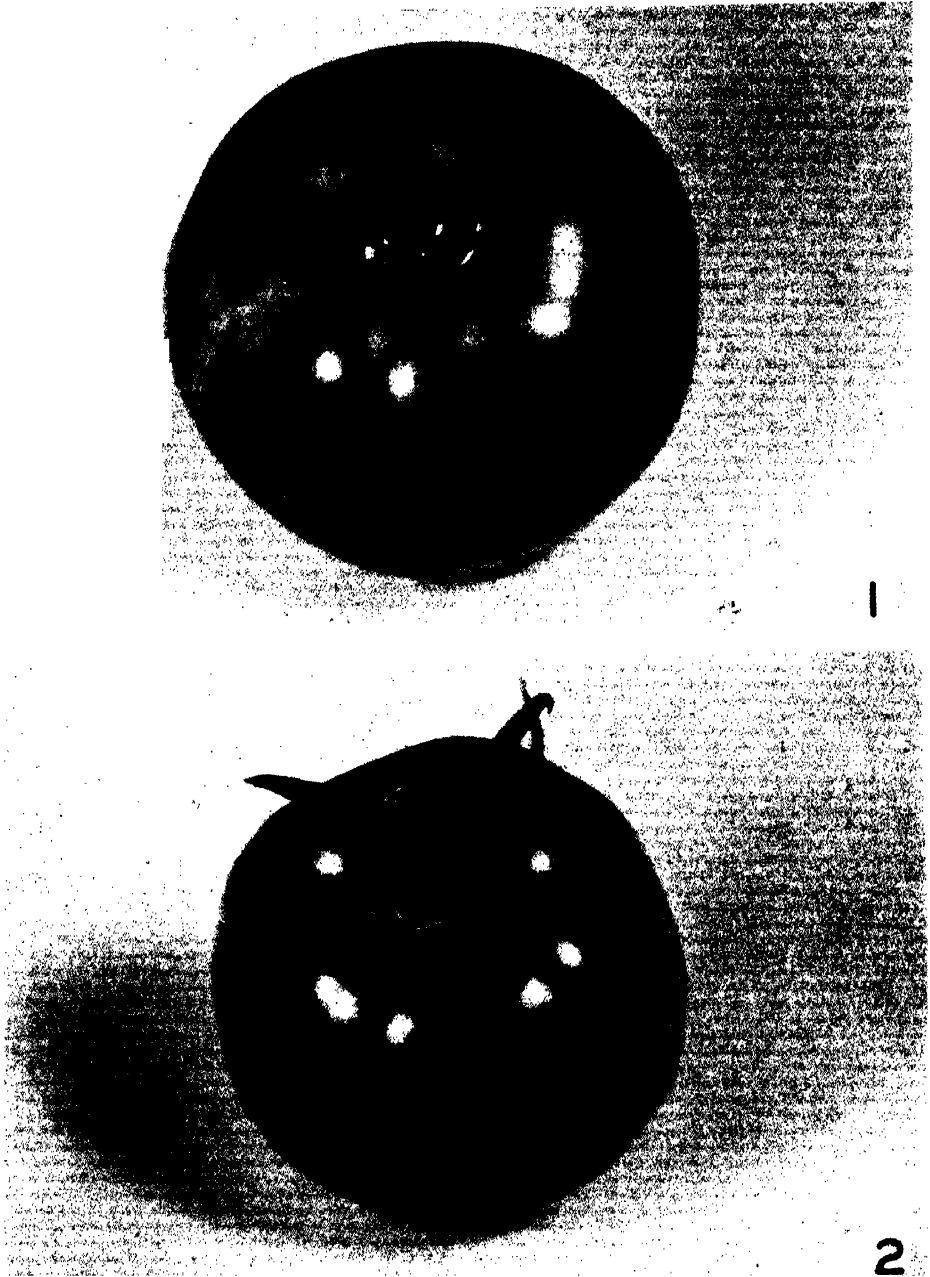


FIG. 1. Waxed Marglobe fruit, showing a typical breathing pore (arrow) over the annular lenticel.

FIG. 2. Unwaxed Marglobe fruit, showing white sepal scar zone at the base of the pedicel (arrow), surrounded by darker lenticel tissue.





observed in the outer and interlocular walls after the fruits attained a diameter of approximately 1 cm. Moreover mitotic figures were not observed in sections of the placenta and pericarp from two- to three-weeks-old fruits.\*

These observations are in general agreement with those of Smith (14), which were made on the Bonny Best variety. His data differ from those of Table II with respect to the final depth of cells composing the pericarp. This is not believed to be a real varietal difference for the following reasons: the camera lucida drawings by MacGillivray (11, Fig. 6) of Bonny Best fruit tissue show the same depth of cells in the mature fruit wall as are recorded above for the Grand Rapids variety; examination of Smith's drawings (14) reveals that his cell counts were taken through the vascular bundles rather than between them as in the present study.

Houghtaling's conclusion (8) was based on the direct proportionality that was found to be maintained between cell volume and fruit volume throughout the grand period of growth. Her data (Fig. 2, p. 249) show, however, that this simple relationship of cell size to fruit size ( $k = 1$ ) does not appear until approximately one week after setting. It therefore appears that among all varieties of *Lycopersicon esculentum* that have been investigated, cell division normally persists in the ovary wall for several days after pollination, and that subsequent growth is entirely by cellular enlargement.

#### *Laggard Fruits*

Tomato plants often bear a few fruits that grow very slowly, reach a final diameter of 15 to 30 mm., and ripen at an age of about 60 days. Our observations on approximately one hundred of these fruits (Grand Rapids and Marglobe) indicate that they are always seedless, and that their locular pulp does not remain green during ripening as in the larger parthenocarpic fruits obtained with growth chemicals (*vide infra*). When fully ripened, these stunted fruits have a dull appearance, lacking the luster of normal fruits. Undersized parthenocarpic fruits of the Grand Rapids variety, obtained by smearing ovaries with 0.5 to 1.0% indolebutyric acid in lanolin, had the same external appearance when fully ripened. The number of cell layers in the pericarps of fruits from the naturally seedless class was found to be the same at maturity (14 to 16 layers) as exists in ovaries at the 'setting' stage. The factors responsible for their seedless habit and reduced size thus affect cell division, possibly after setting.

#### *Effects of Growth Chemicals on Fruit Yield and Quality*

The experiment reported in Table III was designed to test the effects of spraying pollinated flowers, in late spring, with a growth chemical solution that had previously proved highly satisfactory in early spring applications when natural setting was very poor (9). The two varieties used in the present work differed in their natural ability to set fruits. Under favorable conditions,

\* Truscott, J. H. L. *Unpublished observations.*

TABLE III

NUMBER AND WEIGHT OF FRUITS OBTAINED FROM HORMONE SPRAYED  
AND UNTREATED TOMATO FLOWERS

Fifty flowers per treatment

Treatment	Number of fruits set	Average wt. of fruits, gm.	Total weight of fruits, gm.
<i>Grand Rapids variety</i>			
Pollinated (control)	30	58.5	1756
Pollinated and sprayed	33	55.5	1833
Emasculated and sprayed	9	44.1	397
<i>Marglobe variety</i>			
Pollinated (control)	9	89.7	807.5
Pollinated and sprayed	25	147	3675
Emasculated and sprayed	20	110	2196

the Grand Rapids variety sets fruits abundantly. Although Marglobe is one of the most important canning and shipping varieties grown in the United States (13), its unreliability with respect to fruit setting is a serious shortcoming (1).

All fruits from the control Grand Rapids flowers contained large numbers of seeds in each locule. Spraying pollinated Grand Rapids flowers reduced the number of fruits containing seeds, but increased the total number and total weight of fruits obtained. The average weight of individual parthenocarpic fruits obtained from either pollinated or emasculated Grand Rapids flowers was less than that of the fruits containing seeds. As a result of this spray treatment, few of the emasculated Grand Rapids flowers bore fruit of marketable size.

The Marglobe variety was characterized by lower natural setting, and higher artificial setting of emasculated and pollinated flowers than was observed in the adjoining Grand Rapids population (Table III). The final size was consistently greater for the parthenocarpic Marglobe fruits obtained from emasculated flowers than for Marglobe fruits containing seeds. The majority of the fruits obtained from Marglobe flowers that had been both pollinated and sprayed were without seeds, and the remainder contained approximately one seed per locule. The yield of fruits from pollinated Marglobe flowers was increased approximately four times by spraying, despite the lateness of the season and restriction of the treatment to hand-pollinated flowers of middle trusses. With this variety at least, the yield may be improved by the use of growth chemicals, even when the light conditions are favorable for the production of viable pollen.

The parthenocarpic fruits were indistinguishable in external appearance from the fruits containing seeds and neither class showed appreciable 'puffiness'. The seedless and normal fruits showed equal numbers of cell layers in the pericarp, which indicates that mitosis after setting was not affected by the growth chemicals. The parthenocarpic fruits of both varieties were characterized by the retention of green color in the locular pulp during vine ripening. The ripening color change in fruits containing seeds usually begins in the locular pulp before it is shown by the exposed parts. The parthenocarpic condition seems to reverse the ripening sequence, i.e. from the outside in, rather than from the inside out. Microscopic examination of the locular pulp of ripened and partially ripened parthenocarpic fruits revealed that the green color is largely imparted by intact chloroplasts. These are particularly numerous around the free ends of the vascular strands that normally serve the seeds.

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# THE MINERAL REQUIREMENTS FOR FLUORESCIN PRODUCTION<sup>1</sup>

By JOYCE V. KING,<sup>2</sup> Jack J. R. CAMPBELL,<sup>3</sup> AND BLYTHE A. EAGLES<sup>4</sup>

## Abstract

An investigation of the salt requirements for fluorescin production has been carried out, and the mineral balance for maximum fluorescin production determined. Employing ammonium succinate as a source of nitrogen, the ions  $\text{PO}_4$ ,  $\text{Mg}$ ,  $\text{SO}_4$ , and  $\text{Fe}$  have been found essential to the formation of the pigment. Optimum production of fluorescin resulted when 0.01% magnesium chloride, 0.06% dipotassium hydrogen phosphate, 0.005% potassium sulphate, and 0.000005% (0.05 p.p.m.) ferric chloride were used.

The influence of high concentrations of  $\text{Mg}$  and  $\text{SO}_4$  ions in the presence of varying concentrations of iron with ammonium succinate as the source of nitrogen on the formation of both fluorescin and pyocyanin has been determined.

Fluorescin production has been demonstrated to depend primarily upon the concentration of sulphate, iron, and magnesium in the medium. The concentration of iron required has been shown to be of paramount importance. Increasing the amounts of either  $\text{SO}_4$  or  $\text{Mg}$  did not bring about the pronounced stimulation of fluorescin production as was evidenced in the case of pyocyanin.

## Introduction

In studies on the amino acid and mineral requirements for pyocyanin production by *Pseudomonas aeruginosa*, the required salt balances for maximum production of pyocyanin employing specific amino acids as a source of nitrogen have been established (2 and 3). Although certain conclusions could be drawn with respect to the conditions governing the production of bacterial fluorescin no attempt was made to investigate thoroughly the factors relating to the formation of this green fluorescent pigment.

Various media for the production of the pigment have been proposed. In his work on pyocyanin formation Auel (1) noted that a fluorescent pigment was produced by *Pseudomonas pyocyaneus* but thought its formation to be a caprice of the organism. However, Sullivan (6) contended that an alkaline reaction and high phosphate content favored fluorescent pigment production while low phosphate and an acid reaction favored pyocyanin formation. In 1932 Robinson (5) described a synthetic medium for fluorescin production and in 1942 Jamieson (4) showed that the ions  $\text{SO}_4$ ,  $\text{Mg}$ , and  $\text{PO}_4$  are essential to its production.

The object of the present investigation was to establish the conditions optimum for fluorescin production in the absence of pyocyanin formation and in the light of the data thus obtained to clarify the behavior of the organism with respect to the formation of both pigments.

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## Methods

The organism employed in this work was *Pseudomonas aeruginosa* A.T.C. 9027.

In order to determine the influence of the respective constituents of the medium on fluorescin production a series of flasks were prepared, the contents of which differed only by the concentration of the variable under test. For the preparation of media concentrated stock solutions of the various constituents were prepared and preserved under toluene. Basal media of twice the final concentration were prepared, the variable constituent was added, and the medium was then made to volume with water. All media were made up in 10 ml. quantities in 125-ml. Erlenmeyer flasks. Reagent chemicals were used and all solutions were made up with water redistilled through glass. The 125-ml. Erlenmeyer flasks were covered with 100-ml. beakers. Glassware was thoroughly treated with aqua regia and rinsed with water redistilled through glass. Media were sterilized at 15 lb. pressure for 15 min.

The inoculum was prepared by transference of a loopful of a 24 to 48 hr. culture of the test organism into 10 ml. of Sullivan's medium and incubation at 30° C. until the appearance of faint turbidity—usually about 24 hr. The cells were then centrifuged and resuspended in 10 ml. of sterile physiological saline.

The extent of fluorescin production was determined by means of a fluorimeter employing a 1 : 25 dilution of the chloroform insoluble fraction of the medium in calibrated tubes. Dichlorofluorescin was used as standard. Pyocyanin was measured as previously described (3).

## Experimental

In studies on the effect of the nitrogen source on fluorescin production the basal medium consisted of 0.1% dipotassium hydrogen phosphate, 0.1% magnesium sulphate septahydrate, 0.05 p.p.m. ferric chloride, and 1.0% glycerol.

In studies on fluorescin production uncomplicated by the elaboration of pyocyanin it is essential that the amount of iron added to the medium be kept at a low level, approximately 0.017 p.p.m. (0.05 p.p.m. ferric chloride); the amount of iron present in the medium from other sources is estimated to be 0.009 p.p.m.

When using, as the source of nitrogen, potassium nitrate, sodium nitrate, or ammonium sulphate at a concentration of 0.02 moles of nitrogen per liter, fair growth was obtained but there was no evidence of pigment formation even after four days' incubation in these media.

The ammonium salts of various dicarboxylic acids were employed as sources of nitrogen—oxalic, malonic, succinic, glutaric, adipic at a concentration of 0.025 moles of nitrogen per liter, respectively. In this series the dipotassium

hydrogen phosphate and magnesium sulphate were employed at 0.05% concentration. Growth was obtained in all media but in the case of ammonium oxalate no pigment was formed.

When the determinations were carried out after two days' incubation the pigment formation from ammonium succinate was greater than that observed in the other sources of nitrogen; after four days no difference in the amount of pigment formed in the various media was observed.

Ammonium succinate was shown to be the most suitable source of nitrogen for the development of fluorescin, and higher concentrations were shown to be without significant influence on pigment production. A gradual lessening in the amount of pigment formed proportional to the variation in chemical structure from ammonium succinate was observed.

For the determination of the comparative effect of ions essential to fluorescin production media containing varying concentrations of dipotassium hydrogen phosphate, magnesium chloride, and potassium sulphate were prepared. The salt concentrations employed and the amounts of fluorescin and pyocyanin obtained from the respective media are recorded in Table I.

TABLE I

THE EFFECT OF SALT CONCENTRATION ON FLUORESCIN AND PYOCYANIN PRODUCTION

K <sub>2</sub> HPO <sub>4</sub> , %	MgCl <sub>2</sub> , %	K <sub>2</sub> SO <sub>4</sub> , %	Fluorescin*	Pyocyanin**
0.05	0.01	0.0004	0	0
0.05	0.01	0.0005	Trace	0
0.05	0.01	0.002	18	0
0.05	0.01	0.008	18	0
0.05	0.01	0.01	18	0
0.05	0.01	0.1	17	0
0.05	0.01	0.2	14	0
0.004	0.01	0.005	12	3.5
0.008	0.01	0.005	13	4
0.03	0.01	0.005	18	0
0.08	0.01	0.005	19	0
0.1	0.01	0.005	17	0
0.05	0.001	0.005	0	0
0.05	0.004	0.005	14	0
0.05	0.01	0.005	18	0
0.05	0.05	0.005	12	0
0.05	1.00	0.005	0	0

In Table I the amount of fluorescin formed is shown to depend on the concentration of dipotassium hydrogen phosphate. At lower concentrations of phosphate with resulting diminished conditions of growth the low concentration of iron in the medium appears to be released for the formation of small quantities of pyocyanin.

\* Fluorescin expressed in terms of percentage of dichlorofluorescin  $\times 10^4$ .

\*\* Pyocyanin expressed as reading on logarithmic scale of Fischer Electrophotometer.

Magnesium is shown to be essential for fluorescin production, which is seen to depend upon the concentration of this ion in the medium, Table I. As will be shown later, Table III, the influence of magnesium on fluorescin production is in a measure determined by the relative concentration of the sulphate ion.

Although the sulphate ion is not required for growth its presence in the medium is essential for fluorescin production. The optimum concentration would appear to lie within the range of 0.002 to 0.01% potassium sulphate.

In order to determine the optimum concentrations of the respective salts required for fluorescin production, media containing these salts at varying concentrations were prepared. The results obtained are recorded in Table II.

TABLE II  
OPTIMUM SALT CONCENTRATIONS FOR FLUORESCIN PRODUCTION

K <sub>2</sub> HPO <sub>4</sub> , %	MgCl <sub>2</sub> , %	K <sub>2</sub> SO <sub>4</sub> , %	Fluorescin	Pyocyanin
0.03	0.01	0.005	18	0
0.05	0.01	0.005	19	0
0.06	0.01	0.005	20	0
0.08	0.01	0.005	19	0
0.03	0.16	0.005	17	0
0.03	0.010	0.005	18	0
0.03	0.008	0.005	17	0
0.03	0.005	0.005	16	0
0.03	0.01	0.003	19	0
0.03	0.01	0.005	19	0
0.03	0.01	0.008	17	0
0.03	0.01	0.01	15	0

The production of fluorescin is seen to be maintained at a high level over a wide range of concentrations of phosphate, magnesium, and sulphate ions. On the basis of these findings, a medium most favorable to fluorescin production was evolved as follows:

Ammonium succinate.....0.03%  
 Glycerol.....1.0%  
 Ferric chloride.....0.05 p.p.m.  
 Magnesium chloride.....0.01%  
 Dipotassium hydrogen phosphate.....0.06%  
 Potassium sulphate.....0.005%

In the work of Burton *et al.* (2) it was shown that either the magnesium or sulphate ion may in a measure substitute for one another in a medium designed for maximum pyocyanin production in which amino acids serve as the source of nitrogen. In the light of the findings reported herein in which ammonium



succinate was successfully employed as a source of nitrogen, it was considered desirable to determine whether or not results comparable with those reported by Burton *et al.* would be obtained. With this end in view the experiments detailed in Table III were carried out under conditions favorable to pyocyanin formation without fluorescein production. Parallel experiments on fluorescein formation in the absence of pyocyanin production were also performed. The results are recorded in Table III.

TABLE III

THE EFFECT OF HIGH CONCENTRATIONS OF MAGNESIUM AND SULPHATE IONS ON PIGMENT FORMATION

MgCl <sub>2</sub> , %	K <sub>2</sub> SO <sub>4</sub> , %	(High iron—10.0 p.p.m. FeCl <sub>3</sub> )		(Low iron—0.05 p.p.m. FeCl <sub>3</sub> )	
		Pyocyanin	Fluorescein	Pyocyanin	Fluorescein
0.5	0.005	35	0	0	15
0.5	0.18	79	0	0	13
0.5	0.6	94	0	0	13
0.1	1.0	57	0	0	18
0.3	1.0	100	0	0	17
1.0	1.0	85	0	0	0

The findings of Burton *et al.* with respect to pyocyanin are confirmed. In the case of fluorescein, increasing the concentrations of sulphate ion did not exhibit the pronounced effect on pigment production observed for pyocyanin. With magnesium, similar results were obtained within certain concentrations of the ion. Above 1% magnesium chloride complete absence of fluorescein production was observed even though marked growth occurred.

In the light of the findings of Burton *et al.* on the interrelationships of magnesium, sulphate, and iron on pyocyanin formation and with the object of lessening the possible influence of trace contaminants present in the high concentrations of salts employed by these workers, experiments were carried out using lower concentrations of these ions. In the presence of an adequate supply of iron, pyocyanin would be produced and in view of the results recorded in Table II in which 0.01% magnesium chloride, 0.005% dipotassium phosphate, and 0.05 p.p.m. ferric chloride provided a good medium for growth and gave high yields of fluorescein, experiments were carried out employing similar concentrations of magnesium and sulphate in the presence of higher quantities of iron. The results of these experiments are recorded in Table IV.

In Table IV, the results obtained when the concentrations of magnesium and sulphate were varied over a considerable range with the concentration of iron held at a uniformly high level of 0.001% (10 p.p.m.) ferric chloride are detailed.

Good growth was obtained in all media. Fluorescein was absent under all conditions thus confirming previous observations. The results show clearly that pyocyanin formation depends primarily upon the quantities of sulphate

and iron present in the medium and that although magnesium is required for pyocyanin formation its essential function is concerned with the growth of the microorganism.

TABLE IV

THE ROLE OF MAGNESIUM, SULPHATE, AND IRON IN PYOCYANIN PRODUCTION

MgCl <sub>2</sub> , %	K <sub>2</sub> SO <sub>4</sub> , %	FeCl <sub>3</sub> , %	Pyocyanin	Fluorescin
0.01	0.005	0.001	0	0
0.01	0.03	0.001	18	0
0.01	0.05	0.001	33	0
0.02	0.005	0.001	0	0
0.1	0.005	0.001	0	0
0.3	0.005	0.001	0	0

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# SEEDS AND SEEDLINGS OF THE GENUS *BRASSICA*<sup>1</sup>

By JEAN M. MCGUGAN<sup>2</sup>

## Abstract

The genus *Brassica* with its numerous cultivated strains and weed varieties presents a difficult problem to the seed analyst. A solution is attempted in this paper. Peculiarities of seed color, shape, and histology have been used wherever possible as diagnostic characters. In cases where these were found to require verification the characters of the young seedlings were used to supplement them. It has been found possible to distinguish the seeds or seedlings of plants so closely related as to be separated in adult form only by differences in the depth of the yellow color in their roots and blossoms. Care has been taken to test the material for authenticity and to make sure that the seed and seedling characters chosen are closely linked in heredity with distinguishing characters of the species and varieties. A key for the identification of seeds of known varieties of *Brassica* has been evolved.

## I. Introduction

The term "Brassica" was used by the Greeks as early as 200 B.C., and was first applied to the cabbage group of plants, *Brassica oleracea* L., together with the radish, *Raphanus*—plants which indeed had already been in use for several hundred years. It is thus noted in Cato's "De Re Rustica", 149 B.C., but, since then, its scope has gradually broadened and it has now become a genus of widespread economic importance, ranging from the much desired foodstuffs of animal and man to some exceedingly undesirable and more or less poisonous weeds, and is probably the most outstanding genus of the Cruciferae.

Linnaeus, in his eighteenth century classification, distinguished from the genus *Brassica* a genus *Sinapis*, which was originally intended to represent the true mustards. It was found, however, that mustard principles were prevalent throughout, and as they varied in concentration from that sufficient to add piquancy to salad greens to that adequate to supply condiments, counterirritants, and rubefaciants for commercial use, they formed only a relative basis of classification. During the nineteenth century there was a concerted movement to eliminate the genus *Sinapis* and include all of its members in the genus *Brassica*. This later classification is commonly followed in Canada. Engler's "Das Pflanzenreich", however, in the section on Cruciferae-Brassicaceae compiled by O. E. Schulz in 1919, still retains the Linnaean classification but bases the distinction on the lateral nerves of the fruit. In his work 35 species are placed in the genus *Brassica*, while *Sinapis* comprises seven of which two are *alba* and *arvensis*. It might be mentioned here that Kamenskei

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(1931 (28) ) claims that in the seed coat of the genus *Brassica* the large cells and the epidermis compress into one layer while in *Sinapis* the epidermis is distinct. The writer has not found that this distinction is valid in all cases and in the present work all species are taken as belonging to the one genus, *Brassica*.

As might be expected, a genus as large as this one is represented by a variety of forms, but the genus *Brassica* exhibits some unusual modifications. Worthy of special note is the fact that either the flower, the shoot, the stem, the root, the leaves or parts or combinations of these may develop characteristically while the remaining vegetative organs of the plant remain normal. This development is marked in *B. oleracea* L. . In the variety *botrytis* L., the whole flower structure, but principally the peduncle, enlarges to form the cauliflower or, in a later strain, the broccoli head. In the variety *capitata* L., the stem becomes so dwarfed that the leaves fold over each other in a solid cabbage head. In the variety *gemmifera* DC., Brussels sprouts, individual shoots form themselves into small heads in the axils of the stem leaves. In the variety *caulorapa* DC., kohlrabi, the stem becomes swollen, and in the variety *acephala* DC., the kales, the leaves and the stem may become slightly swollen, or the leaves become variously cut and curled. Similar tendencies are seen in other species. In *B. pekinensis* Rupr., pe-tsai, the petioles and veins of the leaf become variously modified. In *B. napobrassica* Mill., rutabaga or Swede turnip, the lower part of the stem and the root form themselves into an enlarged schizocorm, and in *B. Rapa* L., the turnip, the root alone becomes enlarged. In many forms, however, modifications are not apparent or, if present, are only relative and not distinct.

Cause for these modifications may be found in the following contributory factors: (1) within the genus variations frequently occur and polymorphism is common, (2) improvement of type through selection is constantly being maintained, and (3) species crosses, as well as intergeneric crosses, are possible, and many variety crosses occur, as Kristofferson (1924 and 1927 (33, 34) ), Kakizaki (1925 (27) ), Karpetchenko (1924 and 1927 (29, 30) ), Sinskaja (1927 (58) ), and Malinowski (1929 (39) ) have shown. Independent or multiple hereditary factors may be involved. Red color of cabbage, for example, as well as certain other color units, behaves as a simple Mendelian character, whereas the hearting of cabbage, the curling of the kale leaf, and the shaping of the storage organs are the result of multiple factor groups. The complication of the picture may be increased by complete or partial linkages, as well as multiple linkages where the individuals from two groups of multiple factors are themselves linked.

On the other hand two species that have followed different lines of development may contain certain identical factors and in consequence show surprising similarities. Note the striking resemblance between the enlarged storage

organ of certain strains of rutabaga and turnip (Kajanus, 1917 (26) ). Considering that these two plants belong to different species, have different colored flowers, and a different number of chromosomes (*B. Napus*  $2n = 38$  and *B. Rapa*  $2n = 20$ , Howard, 1940 (24) ), this likeness might easily cause confusion. Conversely allelomorphic factors are also to be found in these two species, the hairiness of the rutabaga leaf and the smoothness of that of the turnip (Malinowski, 1929 (39) ).

Considering that it is on distinctions of the above order that plant classification is necessarily based, one need feel no surprise that different systematists have differed so radically in their *Brassica* groupings; the resultant confusing nomenclature of the genus\* is, however, unfortunate.

In the light of these facts consider the possibility of identifying *Brassica* seeds without destroying them, i.e. by the outer seed coat marking, or the color, size, and shape of the seed itself. Such an identification presupposes that, in seed of each variety, one at least of the above characters is controlled by simple factors completely linked with some factor or factors responsible for the particular identification features of that variety of plant. Undoubtedly many such incidents exist but in certain cases they are lacking.

Another difficulty presents itself to one attempting the identification of *Brassica* seeds by inspection alone. Whether due to multiple factor groups or to some other cause the seeds of one variety quite commonly show a gradation of certain characters that may extend into one or two closely related varieties. This is so general that *Brassica* seeds in common use seem to form themselves into a practically unbroken series from *B. oleracea* through *B. Napus*, *B. napobrassica*, *B. Rapa*, and *B. campestris* to *B. juncea*. Of course there are exceptions to and minor offshoots from this series but if enough varieties and strains are on hand it is surprising how gradual the transition appears. Granting that many of these varieties are clear-cut and distinct and will always remain true to type, there are others which, even when their seeds in bulk lot can be placed in one variety or another, will contain some individual seeds whose diagnosis is by no means clear.

Turning to the literature on the subject, we find that the first studies of the seeds of the genus *Brassica* were undertaken by pharmacologists and at first centered on the hilum and micropyle. Later, and until the end of the nineteenth century, the main efforts were directed toward discovering the layers that make up the seed coat. In the twentieth century work turned to the distinction of *Brassica* seeds by their very young seedlings, and thus naturally pigments were brought into the limelight. This was followed by a study of older cotyledons, and later, of quite well developed plants, with attention being directed to the character and shape of cotyledons and first

\* For a most interesting account of the derivation of *Brassica* names I would make reference to: "*Flora of Middle Europe*", by Gustave Hegi, 1906.

leaves. Some work was done with the use of ultraviolet light. In this literature there is a confusion of scientific names coupled with common names in unfamiliar languages. It thus happens that it is often impossible to take full advantage of the work that has been done, because unfamiliarity with the common varieties of the countries concerned makes it difficult definitely to connect their variety name with ours.

This paper which treats on different phases of *Brassica* identification in turn, is the result of investigation extending over a number of years, during the course of which it has been found that generally speaking when *Brassica* plants themselves are distinctive their seeds may be conclusively identified. On the other hand where the distinction between plant groups is based merely on relative modifications, a corresponding correlation exists with reference to their seeds, which can be distinguished only by a complete study of the seed and seedling from all angles and a careful evaluation of the results.

For such an investigation a broad selection of authentic samples of seed is necessary and in the present work more than seven hundred such samples were used. Of these 85 came from the Central Experimental Farm, Ottawa, where their identity had been certified. Through the courtesy of W. H. Wright, Chief of Analytical Services, Ottawa, I was enabled to study five samples of rape seed identified by Dr. F. H. Hillman, former Assistant Botanist, Seed Laboratories, Washington. The rest of the seeds were obtained from various seed companies, botanical gardens, and government stations at home and abroad. From the majority of these samples plants were raised to maturity and identified as to variety. In this connection my grateful thanks are due to Mr. A. Hope of the Canadian Seed Laboratories at Sackville, N.B., as well as to the Forage Crop Division, Ottawa, for the growing and forwarding of plants of 90 of them. The remainder were grown by the writer who is indebted for aid in their identification to Dr. L. H. Bailey, Cornell University, who very kindly allowed her to study the plants in his herbarium.

Grateful acknowledgment of valued assistance and advice is due to Dr. H. B. Sifton under whose direction this work has been carried on. I wish to thank the members of the staff of the University of Toronto, mentioning in particular Miss C. B. Ross for help with drawings, Dr. Gertrude Wright for constructive criticism, Dr. D. Hamly for aid in photography, also members of the staff of the Plant Products Division, Department of Agriculture, Canada, in Toronto and Ottawa, who have contributed to the work.

## II. Seed Coats

### (Their Importance in Seed Identification)

A very thin radial section of the *Brassica* seed coat can be used to identify species and very often varieties. Typical *Brassica* seeds are fairly easily placed but those that are off type require careful study. It should first be

noted that the macroscopic appearance of the seed coat is valuable in the elimination of certain varieties. The thin coat of Brussels sprouts, for example, contrasts definitely with the thick heavy one of marrow kale. Secondly, portions of the seed coat, cleared in chloral hydrate or weak potassium hydroxide, often show many of the same characters as the radial section and are much more easily made available for study. This is particularly useful in observing palisade cells.

In the study of the seed coat the epidermal layers were first noted. Oudemans (1854 (45)) seems to have been the first to interpret the tumbler cell of the palisade layer and he described also a subepidermal layer. Fluckiger (1867 (15, p. 687)) was confused about the palisade and pigment layers while Berg (1865 (3, p. 92)) had only three layers to the coat and these were not clear. Schröder (1871 (55)) embedded seeds in wax for cutting and mounted the sections in glycerine. Tietscher (1872 (61, p. 72)) had several illustrations and noted a layer with intercellular spaces below the epidermis. Fluckiger and Hanbury (1874 (16, p. 141)) used turpentine for mounting and showed closed palisade cells and a pigment layer with firm, solid-walled cells. Sempelewski (1874 (56)), like Schröder (1871 (55)), and at a later date Hartz (1885 (21)), studied seed tissues. He could not distinguish *B. Rapa* and *B. Napus*, contradicted the statement that intercellular spaces are present below the epidermis, and has one of the best of the earliest papers. Hohnel (1875 (23)) also used Schröder's method of embedding in wax in a small hole in a cork for cutting, treated his sections with caustic potash, nitric acid, or Schultze's maceration fluid as desired, and stained with chlorzinc iodide. He distinguished *B. Napus* from *B. Rapa* by the ratio of the length and breadth of the palisade cells, which were uniform in height in *B. Napus* but not in *B. Rapa*. He decried the idea that a study of ontogeny was indispensable in the interpretation of the seed coat. Hartz (1885 (21)) claimed that the lumen of the palisade cells was greater than the double wall in *B. Napus* but not in *B. Rapa*. Nobbe (1887 (43)) did not clearly distinguish separate coats and Wittmack, the same year, investigated oil seeds.

Investigation turned to endosperm tissue and D'Arbaumont (1890 (1)) claimed to have found a new coat adjacent to the aleurone layer that was a remnant of the nucellus not changed into aleurone tissue. Others, such as Cauvet (1888 (9)), Brandza (5), Poisson (50), and Maury (1889 (40)), and Atterberg (1899 (2)), disagreed as to the number of layers in the seed coats, these ranging, in their opinions, from three to six. Guignard (1893 (19)) found that the so-called air spaces beneath the epidermis were merely large cells. This was the beginning of the "Grossencellen layer" to which so many references are later made. Burchard (1894-1896) made careful illustrations of radial sections of 12 species. His drawings repay careful study. Pieters and Charles (1901 (49)) claimed to have found differences in the coats of

*B. oleracea*, *Napus*, *nigra*, and *arvensis* but none between Swede turnip and rape. Böhmer (1903 (4)) and Winton (1906 (64)) described individual seeds. Wisselingh (1919 (65)) explained with illustrations the development of the coats from the integuments and nucellus in *Brassica nigra* and *alba*, *Cheiranthus*, and *Cochlearia*. He states that sometimes the inner cuticle, between the inner integument and the nucellus, remains and becomes a "cork layer" dividing the pigment layer from the endosperm. Viehoveer, Clavinger, and Ewing (1920 (63)) described Chinese colza found in America. Muravieva (1928 (41)) illustrated head cabbage, Swede turnip, two varieties of turnip, brown mustard, and wild mustard. Her outer parenchyma layer seems to be what this paper calls the outer subepidermal row, and she has drawn all species with transparent intercellular spaces above the lignified cups, having missed the thin walls that limit the palisade cells in this region. Roberts and Thomas (1933 (52)) have outlined a key of radial sections of some Cruciferae. They based their first distinction on the species *alba*, *arvensis*, and *nigra* having epidermal cells "containing a large amount of visible mucilage", and another on the absence of a subepidermal layer in *B. rapa* L. and *B. napobrassica* Mill. With these as distinctions I found it impossible to agree. A paper by Orechova and Lapinskaja (1936 (44)) picturing radial sections of varieties of *B. oleracea* should be noted. They distinguished red cabbage from white by the irregularity of the outer row of cells in the seed coat of the former. The anthocyanins in the seven-day-old seedling would seem to be a simpler means of distinction.

The effect of ultraviolet light on *Brassica* seeds was investigated by Chmelar (1925 (10)) and Gentner (1920 (17)). Ferguson and Ashe (1928 (14)) used polarized light on scrapings from the outer layer of the seed coat. They found that the mucilaginous substances were optically active in varieties of *B. oleracea* L. but not in other forms.

This completes the rather voluminous literature on *Brassica* seed coats in so far as it is known to the writer. It indicates that in the structure of the seed coats we have a means of identification useful in some varieties though not in all. However, the use of various unidentified variety names by authors in different countries and the lack of agreement between workers as to various details of seed coat histology make a complete single investigation of known varieties necessary, if such characters are to be used to full advantage.

Such an investigation has been made, and from it the following conclusions found to be of practical taxonomic use have been drawn.

The radial section of *Brassica* seed coat has the following layers, the distinction of which may be more clearly understood by reference to Fig. 16, where all layers are clearly distinguishable.

### 1. *Epidermis*

A single layer of very thin cells, five- to seven-sided in surface view.



## 2. Subepidermal Layer

The layer between the epidermis and the palisade layer, the walls of which become blue when tested for cellulose with iodine and sulphuric acid. In some varieties the outer rows seem to have been crushed or partly crushed by pressure of the maturing seed from within, so that the horizontal walls appear as bands tightly pressed together. In others, the outer row is obviously celled. Each of these cells is filled with a mucilaginous material. In radial section this material is homogeneous in *B. arvensis* (L.) Rabenh., but appears to radiate from a cone-shaped structure formed in the center of the base of the cell in *B. oleracea* L. or from a central concentrated threadlike structure stretching from the top to the bottom of the center of the cell in *B. Rapa* L. *rapifera* Metz., shogoin turnip. In *B. alba* (L.) Rabenh., it forms in concentric rings around a central core. The subepidermal layer is not always stretched tightly over the seed. It may be crushed in folds, undulating or otherwise irregular and is, no doubt, responsible for some minor markings on the outside of the seed coat.

## 3. Palisade Layer

A row of cells whose bases and sides to within a greater or less distance from the top have become thickened and lignified so that they appear as heavy tumblers or mugs. This thickening and lignification occurs on the inside of the cell wall. Above the lignified portion this wall, so thin that even the double wall of adjacent cells appear, in radial section, as a sinuous thread, is short and barely discernible in some species, but elongated and individually conspicuous in others. In *B. nigra* (L.) Koch., for example, its arrangement is almost wholly responsible for the primary net that lies on top of the seed coat. Netting caused by variation in height of the lignified portion of the cell wall occurs in other species but it has more the appearance of being a part of the seed coat. In such seed coats the tallest groups of lignified double walls are responsible for the primary net whereas shorter intermediate groups cause the less conspicuous secondary net apparent in the coat of some varieties.

It is interesting to note trends of variation in palisade structure appearing in the genus. *B. oleracea* L., one of the first of the species if taken as listed in Section IV of this paper, has square tumblers with uniform, thin, straight-sided walls of uniform height, Fig. 4. Uneven, heavier lignification of wall becomes apparent in the tumblers of *B. Napus* L., Fig. 6, and of *B. Rapa* L., Fig. 8. In subsequent species the tendency is for groups of these unevenly thickened tumbler walls to become elongated and with smaller bases form tall cylindrical tumblers with comparatively thick walls. These may be found in *B. campestris* L., Fig. 10 and Figs. 12, 14; *B. juncea* (L.) Coss., Fig. 15; and *B. nigra* (L.) Koch, Fig. 17. The palisade layer of *B. arvensis* (L.) Rabenh., at the end of the series, is made up entirely of very tall cylindrical cells of almost equal height and with walls thickened so that there is practically

no lumen. But while there is this general trend of change, each species has its characteristic cell structure, which may be used to good advantage in the identification of the seed.

#### 4. Pigment Layer

Often appears as a solid layer of pigment. Histologically it is the inner integument. In approaching that portion of the seed immediately surrounding the hilum this layer gradually becomes wider until, made up of several rows of cells, it is many times the apparently normal width of the layer as noted on the opposite side of the seed. In the full grown seed it is difficult to interpret. Fluckiger (1867 (15, p. 687)), Viehoveer, Clavenger, and Ewing (1920 (63)), and Muravieva (1928 (41)) claim that in the plants that they describe this layer is normally one cell thick; Hartz (1885 (21)), Arbaumont (1890 (1)), Pieters and Charles (1901 (49)), Wisselingh (1919 (21)), Winton (1906 (64)), and Douven (1935 (12)) claim it to have one row in some varieties and two or more in others, whereas Hohnel (1875 (23)) and Fedosseyeva (1936 (13)) describe it with more than one row. I would conclude that while pigment color might be noted it is not expedient to use the cell structure of the pigment layer in the distinction of *Brassica* seeds. Fedosseyeva, however, has found that the seeds of "*B. chinensis* L. var. *parachinensis* and *B. juncea* L. var. *sareplana*" have bodies, round from above but oval in cross section, appearing in their pigment layers that are not present in "*B. glauca* Kew". The presence of these bodies in *B. pekinensis* Rupr., chihili, has been observed in my material. As they are not present in *B. Napus* L. they might constitute a distinguishing feature between *B. Napus* L. and *B. pekinensis* Rupr.

The outer aleurone layer of the endosperm has cells that in radial section are more or less rectangular although not always regular. They usually have bulging outer and inner walls. The inner endosperm layers of cells beneath the aleurone layer are crushed and are not figured in this work.

In preparing the radial sections for the present work, the seed was attached by means of paraffin to a wooden block that fitted into a sliding microtome. It was found that the seed was held with sufficient firmness if two-thirds of its thickness projected above the wax. The knife was held obliquely in the microtome and the cutting was done as in the celloidin technique. No water was allowed to reach the seed coat, absolute alcohol being used entirely. The section was transferred to glycerin and mounted unstained in glycerin jelly. In temporary mounts the cellulose was stained blue with iodine and a solution of sulphuric acid (60%) with glycerin (40%).

Following is a summary of distinctive seed coat characters of different species and varieties of the genus *Brassica*. The camera lucida drawings in Figs. 3 to 17 from varieties selected as typical will, it is hoped, serve further to clarify these points. It will be noted that seed coat structure may be used in the distinction of most species but is satisfactory only in the case of a few specific varieties.

## Brassica SEED COATS

	Surface view	Subepidermal layer (radial)	Palisade cells (radial)
<i>B. oleracea</i> L. (general)	No pronounced net because of equal height of tumbler walls and of upper limiting wall of palisade cell. Elevations caused by subepidermal layer might be seen in seed coat but not in prepared slide	Outer row generally celled. Cells of different height but each has cone-shaped projection in middle of base from which mucilage contents of cell seem to radiate	Side walls of tumbler cells straight, lignified, and pigmented, uniform in height and thickness; lumen large
1. var. <i>ramosa</i> (DC.) Alef. Thousand-headed kale	Seed coat thick	Outer row with cells lower than in Fig. 4	Tumbler cells large and heavily lignified
2. var. <i>acephala</i> DC. a. Green or variegated kale	Thin seed coat	Outer row with uniform cells	Tumbler cell walls slightly collapsed and undulating in height. Have right-angled flare at top
b. Blue curled Siberian kale c. Cottagers kale	Seed coat thicker	Outer row with uniform cells	Similar to green kale but upper half of tumbler is larger than lower and double wall more pointed
d. Marrow kale	Thick seed coat	Thick with shallow-celled outer row	Tumbler side walls of the large cells same height, flat brim and base, not quite perpendicular. Upper wall long. Pigment prominent
e. Asparagus kale		No conspicuous cells in outer row	Tumbler side walls narrower, higher, and straighter than in Fig. 4
f. Ragged Jack kale		No conspicuous cells in outer row	Tumbler cells almost square with side walls even, thin, straight, flaring at top at angle of 120°
g. Sheep kale	Thin seed coat	No conspicuous cells in outer row	Tumbler cells uniformly straight, tall, and narrow. Lumen same width as double wall, which is slightly rounded at top
3. var. <i>gemmifera</i> DC. Brussels sprouts Figs. 3, 4	Lumen of tumbler cells large and oval. Epidermis six-sided. Seed coat very thin	Outer row unevenly celled	Tumbler cell walls thin and slightly crushed
4. var. <i>capitata</i> L. Cabbage		Outer row has tall cells. In red cabbage these are uneven	Tumblers, with walls thinly lignified and lumen twice the width of double wall, fit tightly together. Upper palisade wall long. Pigment layer thick

*Brassica* SEED COATS—Continued

	Surface view	Subepidermal layer (radial)	Palisade cells (radial)
5. var. <i>botrytis</i> L. subvar. <i>asparagoides</i> DC. subvar. <i>cauliflora</i> (Gars.) DC.	Thin seed coat	Outer row celled	Tumbler walls thicker and straighter-sided than in Brussel sprouts. Lumen and double wall same width
6. var. <i>gongyloides</i> L. Kohlrabi	Thick heavy coat	Outer row celled	Tumbler cell walls straight, of same height, with right-angled bases and tops. Lumen narrower than double wall
<i>B. alboglabra</i> Bailey		Thick, even, shallow cells in outer row	Indications of grouping of 2 or 3 elongated cells to form net. Tumbler cells like cauliflower
<i>B. pekinensis</i> Rupr.	Note round bodies in pigment layer. These bodies are oval in radial section	Crushed	Palisade cells like <i>B. Napus</i> but taller, straighter tumbler and higher upper wall. Broad lumen wider at top
<i>B. Napus</i> L. Dwarf Essex rape Figs. 5, 6	Fig. 5 is view at top of tumbler cells. Line between the outer and inner limiting cell walls represents the apex of conical thickening. Netting caused by elongated tumbler cells as well as the narrowing of the lumen	Crushed	Tumbler cells broad and angular with a flared and turned back brim. A lignified conical thickening occurs just inside the brim. This along with the one from the next cell forms a crater at their point of junction. Upper palisade wall short. Tumblers slightly higher than broad, have lumen narrower than the double wall
<i>B. Napus</i> L. Annual form	Resembles biennial form	Crushed	Walls taller and thicker with the depression of smaller diameter
<i>B. napobrassica</i> (L.) Mill.	Resembles <i>B. Napus</i>	Crushed	Resembles <i>B. Napus</i> . Lumen of tumbler varies but same width as double wall
<i>B. Rapa</i> L. Turnip Figs. 7, 8	Focusing down from the brim of the tumbler cell the aperture gradually decreases to constancy. Fig. 5 is view halfway down. The middle line is the curved wall in sharp focus while the outer and inner walls are slightly out of focus, one above and one below	Crushed	Tumbler cell wall thinner than <i>B. Napus</i> and thicker than <i>B. campestris</i> . The more gradual thickening below the brim gives a rounded appearance to double wall. Upper palisade limiting wall longer than in any closely related variety
Seven-top turnip		Crushed	Tumbler cell walls thinner than turnip, but like it the tumblers press together at the top and separate near to the bottom. Ridges closer and regular

*Brassica* SEED COATS—Continued

	Surface view	Subepidermal layer (radial)	Palisade cells (radial)
Shogoin, tennoje Japanese foliage turnip		Outer row deeply celled each with a central perpendicular axis from which the cell contents radiate horizontally	
Tendergreen, Komatsu-na Mustard spinach		Crushed	Tumbler cells of uneven height with straight walls of uniform medium width, rounded at base. Lumen same width as double wall in the broad cells but narrower in the tall
<i>B. chinensis</i> (L.) Bailey Celery mustard		Crushed, thick	Tumbler cell of uneven height with thick wall increasing in width toward the almost squared top. No depression
<i>B. campestris</i> L., wild form Figs. 11, 12	Lumen of tall cells of the net is very small but of others it is much larger	Outer row not visibly celled. Dips down into lumen of tumbler cell	Tumbler cells thin-walled of uniform height. Upper palisade lateral wall very short. Resembles <i>arvensis</i> in the packing of tall palisade cells
<i>B. campestris</i> L., annual form Figs. 9, 10	Each cell has small lumen. Netting is caused by height of cell along with subepidermal layer	Crushed	Primary net sometimes the result of a single double wall. Upper palisade wall very short
<i>B. campestris</i> L., biennial form Figs. 13, 14	The crushing together of a few palisade cells giving appearance of a heavy wall, along with the height, responsible for netting	Does not dip down into lumen of tumbler cells	Tumbler cells have thin even walls, wide lumen, and are not crowded together
<i>B. elongata</i> Ehrh.		Celled outer row	
<i>B. juncea</i> (L.) Coss. General view		Celled outer row	Three or four of thin-walled tumbler cells growing taller and crowding together at top cause the primary net. Cutinized layer between pigment layer and endosperm
a. Wild form Fig. 15		Outer celled row unusually high	Double lateral tumbler wall high and pencil-shaped
b. Curled mustard		Outer celled row medium high	Lumen broad at base, narrow halfway up, and broad at top
c. Smooth leaf mustard Fig. 16		Outer celled row very shallow	Conspicuous for the elongated and wavy upper walls of the palisade cells

*Brassica* SEED COATS—Concluded

	Surface view	Subepidermal layer (radial)	Palisade cells (radial)
<i>B. nigra</i> (L.) Koch. Fig. 17		Outer row celled	Shallow tumbler cells of even height. Net is caused by the upper limiting palisade walls
<i>B. arvensis</i> (L.) Rabenh. Wild mustard		Outer row celled	Tumbler cells, tall, narrow, of uniform height and crowded closely together
<i>B. alba</i> (L.) Rabenh.	Walls of epidermal layer are collenchymatously thickened	Mucilage appears as in concentric rings around a circular center	

## III. Seed and Seedling Pigments\*

(Their Importance in Seed Identification)

The four pigment groups that may be utilized in identification of seeds of the genus *Brassica* are the chlorophylls, the anthocyanins, and the carotinoids in the seedlings and the pigments found in the pigment layer or impregnated in the lignified portion of the palisade cells of the seed coat.

The leaf greens of the *chlorophyll* group in both the cotyledons and first seedling leaves are characteristic. The cotyledons of *B. balearica* Pers. and closely related species are very dark blue-green while those of the kales are of characteristic colors. The cotyledons and first seedling leaves of *B. pekinensis* Rupr. are lettuce green, those of colza rapes are bright Rinneman's green, while in *B. nigra* (L.) Koch a typical tint of American green can readily be detected. The practical use of this coloring in identifying seeds may be demonstrated by seedlings grown in the Copenhagen germinator, which, if subject to continuous illumination, will give indication of their inherent cotyledon leaf color within two weeks. The color of the primary leaves is also important in identification of seedlings and is included in the seedling descriptions of Section IV of this paper.

Experimenting with *anthocyanins* has proved that while they have a certain significance, they are not true identification factors. In the first place very few *Brassica* varieties are without white strains. This may be the result of lack of a pigment-forming substance, or the inhibition of pigment formation in the plant. Secondly the amount as well as the intensity of color of the anthocyanins formed in the plant in which they occur is directly dependent on the sugar content of the cell along with the temperature, the moisture supply, and the sunlight to which the plant is subjected. Perhaps low temperature, plenty of direct sunlight, a low moisture supply, and a high sugar

\* The color standard used throughout this work was: Color standards and color nomenclature by R. Ridgway. Washington, D.C. 1912.

content in the cell were responsible for the very high color attributed to certain *Brassica* seedlings by Muravieva (1928 (41) ) and by Orechova and Lapinskaja (1936 (44) ). Dorph-Petersen (1927 (11) ) finds color in the Swede turnip hypocotyl. Korpinnen (1933 (32) ) was unable to detect this color but stated that her seeds were grown inside in poor light. In my experiments it was not noted even when the seedlings grew in sunlight out-of-doors in the autumn, under which conditions some of the kales colored strongly.

The presence of anthocyanins in plants is according to Kajanus (1913 (25) ) controlled by simple factors. He states that in the skin of the purple top Swede turnip bulb there is a factor for light purple in the under part of the bulb and one for dark purple in the skin of the upper storage portion. When both are absent the skin is green. In the turnip bulb there are two independent characters determining skin color, *F*, a factor for green skin, and *P*, a factor for red skin. When *F* and *P* are absent the skin is cream yellow and both are independent of each other.

*Brassica* seeds were germinated between blotters in a dark germinator held at 18° to 20° C. At the end of seven days, as a rule, certain color differences were manifest. These color differences were found to be accentuated by immersing the cotyledons in a 65% solution of lactic acid and leaving them for a time at room temperature. A certain proportion of the anthocyanin pigment is soluble in the cold weak acid and within broad limits will place the plant. Thus considering the *Brassica* species in the order as listed in Section IV of this paper we find purple anthocyanins prevalent in *B. oleracea* L. but diminishing in quantity until in some varieties of *B. Napus* L. no pigments are present. In *B. napobrassica* Mill., which perhaps should precede *B. Napus* L., the color of the acid solution will indicate whether the rutabaga is a purple top, a bronze top, or a green top strain. In *B. Rapa* L. red anthocyanins appear in small quantity, and that generally on injury, but the red pigments become more prevalent in *B. campestris* L. and are decidedly marked in *B. juncea* (L.) Coss. and *B. alba* (L.) Rabenh.

This general knowledge is useful and *could certainly be used by an analyst or grower intimately acquainted with the strains involved*, but definite identification of *Brassica* seeds generally by the use of anthocyanins is dangerous.

Table I gives an indication of the pigments that appear most commonly in the very young *Brassica* seedlings grown in the dark.

The third group of color pigments, the *carotinoids*, is of special importance in *Brassica* distinction. In the cotyledonary leaf of the yellow varieties of *B. napobrassica* Mill., the rutabaga, and *B. Rapa* L., the turnip, are carotinoids not found in any other variety of *Brassica*. They are present in granular form in the plastids in the mesophyll tissue, some portion becoming dissolved in the small oil drops within the cell. Their appearance or absence in the turnip or Swede cotyledonary leaf is governed by the same factors that control flower color and bulb flesh color in these varieties. This will be of great interest to the seed analyst or to anyone such as the geneticist or plant breeder, who appreciates speed in distinguishing very young seedlings.

TABLE I  
SEEDLING PIGMENTS

	Anthocyanins				Carotinoids
	Edge of cotyledon	Hypocotyl	On injury	Lactic acid	Cotyledon
Thousand-headed kale	Possible purple tinge	Possible purple or mauve	Purple	Deeply colored mauve or purple	Lemon yellow, some deeper
Green kales			Purple	Purplish-gray or yellow	Lemon yellow, fading
Asparagus kale				Purplish-gray	Greenish-yellow, fading
Collards		Possible purple			
Sheep kale				Colorless	Light yellow
Marrow kale	Possible purple	Possible purple		Deep mauve	Lemon yellow, fading
Ragged Jack kale				Almost creamy	Light yellow, fading
Cabbage and savory cabbage		Purple possible but rare		Yellow or purplish-yellow	Very deep yellow to pale yellow
Brussels sprouts	Mauve or purple	Possible mauve or purple		Slight or deep purple	Deep yellow to lemon yellow
Kohlrabi	Mauve	Purplish-mauve or purple		Slight or deep purple	Pale yellow
Cauliflower	Rarely colored red	Mauve or purple possible		Pale mauve, pink, or pale yellow	Deep yellow to pale yellow
Broccoli	Rarely colored	Rarely colored		Purple or clear light yellow	Deep yellow to pale yellow
<i>B. alboglabra</i>	Reddish-purple			Clear to reddish-purple	Medium yellow to yellow
<i>B. pekinensis</i>	No visible anthocyanins			Clear	Pale to deep yellow
<i>B. Napus</i>	No visible anthocyanins			Yellowish-green	Shades of yellow
<i>B. Napobrassica</i>	No visible anthocyanins			Possible purple or amber	Naples or chrome yellow
<i>B. chinensis</i>	No visible anthocyanins			Amber	Pale yellow
<i>B. rapa</i>	Color very rare		Red	Yellow	Mat or buff yellow or pale yellow, fading to chalky white
<i>B. campestris</i> , annual form	Possible red	Possible red	Red	Clear straw	Light or deep yellow
<i>B. campestris</i> , biennial form	Possible red	Possible red		Clear straw or rose amber, trace of amber	Deep yellow



TABLE I—*Concluded*  
SEEDLING PIGMENTS—*Concluded*

	Anthocyanins				Carotinoids
	Edge of cotyledon	Hypocotyl	On injury	Lactic acid	Cotyledon
<i>B. juncea</i>					Lemon yellow
<i>B. arvensis</i>	Possible red				
<i>B. alba</i>	Red	Red		Deep red	Pale yellow

As early as 1900 Percival discovered this color difference. Later Hallquist (1919 (20)) and Nilsson (1924 (42)) considered it. They, however, worked in Europe where white-fleshed Swede turnips are common. In Canada where their presence is extremely rare the importance of this pigment as a distinguishing factor is infinitely greater. Out of at least one hundred samples of Swede turnips examined, I have seen only two samples of white-fleshed Swede turnips and they did not represent commercial seed. In the common turnip, white forms are very common but the turnip seed is much more easily identified than that of the Swede turnip wherein the identification of certain strains is so difficult that for reliability with speed, this color test is the only one I know. Without exception it identifies yellow-fleshed Swedes, which in Canada represent the Swede turnip or rutabaga.

Color inheritance in the turnip and rutabaga group has been investigated and complete association has been established between their flower color and their root flesh color (Malinowski, 1929 (39)). Kajanus (1913 (26)) believes that the corresponding characters of turnips and rutabagas are due to identical factors. In the case of *B. Rapa* L. he suggests a factor *M* for white flesh inhibiting yellow. He found that this white flesh is strictly associated with bright yellow color of flower while yellow-fleshed plants have mat orange flower color.

As regards minor differences, Sylven (1927 (60)) describes four tints of yellow flower color in *B. Napus* L. var. *oleifera* DC. that he uses in the  $F_1$  generation as an index in working out problems of inheritance. These served quite well in the  $F_1$  generation but not with  $F_2$  plants.

McMaster Davey (1931 (38)) notes that flesh color in the turnip is governed by simple factors of which that for white color is dominant with probably minor factors governing the intensity of the yellow. In rutabagas he finds two distinct but similar pairs of factors each of which are capable of producing white flesh color even when several factors for yellow are present. He advises the use of flower color in keeping stock true to type as flower color and flesh color are controlled either by the same hereditary factors or by closely connected hereditary causes. In rutabaga, yellow-fleshed plants have Naples yellow flowers; white-fleshed turnips, white-fleshed rutabagas, and rapes have

bright lemon yellow petals; while yellow-fleshed turnips have mat buff yellow petals, which, however, are more chalky in appearance than their counterpart in rutabagas.

Thus it is agreed that the factors that control flower color in the turnip and rutabaga control flesh color in these plants, and my results of experiments carried on previous to 1932 suggest further that these same factors also determine pigment formation in the cotyledonary leaves.

In the present work on pigments, the seeds of different strains of rutabaga, turnip, and other *Brassicas* were germinated as for anthocyanin pigments and the seedlings immersed in 65% lactic acid and allowed to stand. Carotinoid pigments are not soluble in the acid, but the latter acting on the cotyledonary leaf tissue has the effect of making it transparent (Simpson, 1929 (57) ), and thus bringing the leaf pigments into prominence. The yellow rutabaga cotyledons changed to a deep cadmium or Naples yellow while the yellow turnip showed a chalky mat orange color. A solution of methyl orange in water of the strength of 3.9 mgm. to a liter gives a fair indication of the rutabaga seedling color. If any doubt exists, seedlings of 8, 9, or 10 days may be used. After 12 hr. the cotyledonary color begins to fade, in some more quickly than in others. For an indication of carotinoid color in the cotyledons of the different varieties of *Brassica* see Tables I and II.

Through the courtesy of the late R. I. Hamilton, of the Central Experimental Farm, Ottawa, I was enabled to compare directly the color of the flesh of 50 strains of turnips and rutabagas with that of the seedling cotyledons grown from the same seed. The cotyledons were immersed in the acid, as were also sections of the bulb flesh. Comparing them it was noted that a distinct division occurred between the white and yellow turnips. It was also evident that just as the flesh color varies even within types so also does the intensity of the yellow and chrome in the corresponding cotyledons. The roots with intensely colored flesh develop from seedlings with cotyledons colored deep cadmium yellow, those with lighter Naples yellow colored flesh from a paler Naples yellow cotyledon while the white Greystones and Milans have a typical rape yellow cotyledon—the yellow varying in intensity with the depth of (cream) color in the root flesh.

A study of the accompanying Table II may serve to illustrate this.

The actual pigments in the rhizocorm of the rutabaga were investigated by Lubimenko (1914 (37) ), who found a small quantity of lycopene as well as an oxidation product of lycopene that he called a lycopinoid. It was more soluble in alcohol and spectroscopically had more intense bands. Tunman and Rosenthaler (1931 (62) ) retained the name lycopinoid but Palmer (1922 (46) ) and Kylin (1927 (35) ) classified under carotinoids both carotene,  $C_{40}H_{57}$ , and its isomer lycopene,  $C_{40}H_{57}$ , with the oxidation products of each. In the flesh of the rutabaga Kylin found carotene and its oxidation product xanthophyll and also lycopene and a red carotinoid that is an oxidation product

TABLE II

## PIGMENT PECULIARITIES IN SWEDE AND TURNIP VARIETIES

Variety	Number	Shape	Bulb pigments			Seedling pigments	
			Skin		Color of flesh	Flesh immersed in lactic acid	Cotyledons immersed in lactic acid
			Top	Bottom			
Milan Earliest Purple Top	3	Flat, round	Purple	White	Greenish white	White	Palest yellow
Milan Early Purple Top	4	Flat	Purple	White	White		Palest yellow
Purple Top Mammoth Improved	673	Round	Purple	Mauve and white	Pure white	White	Pale yellow
Greystone	711	Flat, round	Purple	White	Pure white	White	Pale yellow
Greystone	12	Flat, round	Purple	Creamy white	Pure white	White	Pale yellow
Red Paragon	682	Round	Purple	White	Pure white	(Darkened)	Pale yellow
Pomeranian White Globe	684	Round	White		White	White	Pale yellow
Southern Prize Turnip	15	Round	Mauve	White	White		Pale yellow
Greystone	10	Oval	Purple	White	White	White	Pale yellow
Greystone	674	Round	Bronze	White	White, cream line just inside skin	White	Pale yellow
Cowhorn	14	Long	Bronze	White	White	Creamy white	Yellow
Improved Greystone	678	Oval	Purple		White	Creamy white	Yellow
Purple Top Milan	2	Flat	Purple	White	White	Creamy white	Yellow
Large White Norfolk	706	Flat	Bronze	White	Slightly creamy white	(Darkened)	Yellow
Imperial Green Globe	692	Round	Green		Slightly creamy white	Creamy white	Yellow
Green Globe	694	Round	Green		Slightly creamy white	White	Yellow
White Globe	705	Round	White		Creamy white	Creamy white	Yellow
Centenary Turnip	688	Oval	Cream yellow		Creamy chrome	Creamy chrome	Chrome
Aberdeen Purple Top	671	Round	Purple	Cream	Creamy chrome streaked with white	Clear chrome yellow	Clear chrome

TABLE II—*Concluded*PIGMENT PECULIARITIES IN SWEDE AND TURNIP VARIETIES—*Concluded*

Variety	Number	Shape	Bulb pigments			Seedling pigments	
			Skin		Color of flesh	Flesh immersed in lactic acid	Cotyledons immersed in lactic acid
			Top	Bottom			
Aberdeen P.T. Yellow	8		Purple		Creamy chrome streaked with white	Light chrome	Chrome
Early Sheepfold	686	Oval	Green	Yellow	Pure creamy chrome	Chrome	Chrome
Aberdeen Perfection Green Top	695	Round	Green	Chrome-yellow	Spotted chrome and white	Chrome	Chrome
Yellow Tankard	687	Long, oval	Yellow		Chrome	Chrome	Chrome
Yellow Tankard	715	Round	Purple	Yellow	Chrome streaked with white	Deep chrome	Chrome
Golden Vall	716	Round	Green	Yellow	Deep chrome	Chrome	Deep chrome
Aberdeen Green Top	708	Round	Bronze	Yellow	Chrome	Chrome	Deep chrome
Aberdeen Purple Top	710	Round	Bronze	Yellow	Deep chrome streaked with white	Deep chrome	Deep chrome

of lycopene and which he called arumin. To discover whether these same pigments are present in the yellow strains of rutabaga and turnip, the following investigations were made.

The cotyledonary leaves were nipped off and dried in a low temperature oven at 40° C. To 0.8 gm. of this material freshly ground, 5 cc. of a mixture of 90% petroleum ether and 10% ethyl alcohol was added and the pigments were extracted by boiling. The petroleum ether solution was repeatedly fractionated with 98% aqueous methyl alcohol, and then evaporated. A portion tested with chloroform and antimony trichloride gave the blue color indicative of carotene. The Tswett adsorption method as outlined by Schertz (1929 (54)) was tried on the remaining portion but the carotene solution passed through the calcium carbonate column without being adsorbed.

A comparative test of the pigment from the cotyledons of Orange Jelly turnip, Canadian Gem rutabaga, and Dwarf Essex rape with that of the pulp of the Canadian Gem rutabaga rhizocorm was made. To 0.8 gm. of the dried cotyledons, freshly ground, 5 cc. of 95% alcohol was added and allowed to stand at room temperature over night. The fruit of the tomato

known to be rich in lycopene and the root of the carrot, which contains considerable carotene, were used as check tests. These, as well as the turnip root, were cooked in water until soft. The pulp was then filtered, pressed, quickly washed with 95% alcohol, filtered, and again pressed between filter papers. To 0.8 gm. of this pulped material 5 cc. of 98% alcohol was added as in the case of the seedlings. On the second day the alcoholic solutions were filtered off and 5 cc. of fresh alcohol added. This process was repeated on each of four consecutive days. On the fifth day 5 cc. of ethyl ether was substituted for the alcohol. On the sixth day more ethyl ether was added while on the seventh day any pigment remaining was removed by boiling for 10 min. in absolute alcohol plus a small amount of ether, then adding more ether, and allowing to stand over night. Each day the different solutions were standardized by comparison with lead chromate solutions of different intensities. The results are graphically pictured in Fig. 1. As lead chromate

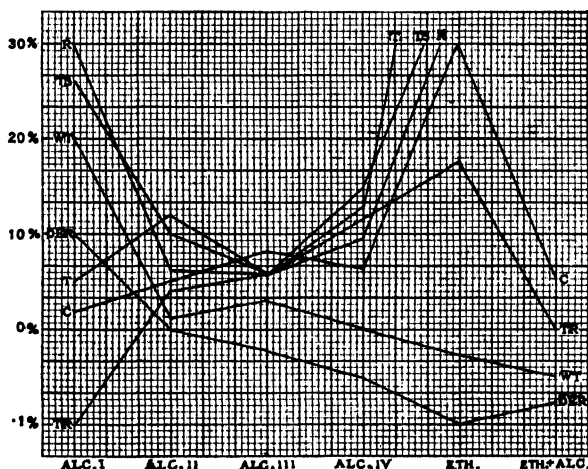


FIG. 1. Abscissa—solvents used; ordinate—percentage by weight of lead chromate solution used in comparison; R.—Canadian Gem rutabaga seedlings; T.S.—Orange Jelly turnip seedlings; D.E.R.—Dwarf Essex rape seedlings; W.T.—White Milan turnip seedlings; T.R.—turnip root pulp; T.—tomato pulp; C.—carrot pulp.

only demonstrates yellow grading into chrome, it is quite inadequate where red colorations appear and thus the ether and alcohol solutions of the tomato and the seedlings of the turnip and rutabaga do not appear on the graph. In the first three alcoholic solutions of all cotyledons, xanthophyll appears. There is also present in the first alcoholic solutions of the cotyledons of yellow turnip and rutabaga seedlings some pigment the color of Naples yellow. This is not present in the white turnip or the rape cotyledons. It is readily soluble in carbon disulphide, appreciably soluble in low boiling petrol ether, and in acetone. These properties along with the color would indicate a lycopene-related pigment.

If the ether solutions from the cotyledons of the yellow turnip and rutabaga as well as those from the tomato pulp are concentrated and evaporated on a microscopic slide, crystals are formed. These appear as fine needle-shaped crystals that are found singly or in sheaths. They are pictured in Fig. 2, and have the typical form of lycopene crystals. Crystals may also be procured by extracting the color from the freshly ground cotyledons with carbon disulphide and shaking with petroleum ether. Redissolve the crystals that form in carbon disulphide and again wash with petroleum ether. When the solution is sufficiently concentrated, there appears, on evaporation, an irregular brownish-red spherical crystal mass from which finer crystals radiate as from a central core. These radiating crystals appear needlelike and are also typical lycopene crystals. On the slide from the ether solution besides the lycopene crystals there is another pigment, amber or reddish-brown in color that does not crystallize out of solution. It is dissolved and tightly held in the small fat globules dissolved from the cotyledon and very closely resembles the arumin found in the rutabaga bulb flesh by Kylin (1927 (35)). In an effort to dissociate it the fat from a carbon disulphide solution of the dried cotyledons was saponified with a 20% alcoholic solution of sodium hydroxide and the pigments separated from the soda solution by adding water and ether in a separatory funnel. The other solution was then washed with twice its volume of distilled water and allowed to evaporate on a micro slide. The pigment was evident but crystals were not formed.

Thus we find in the cotyledons of the yellow turnip and of rutabaga seedlings the same pigments that were present in the adult turnip and rutabaga flesh: carotene, xanthophyll, lycopene, and arumin with more of arumin than lycopene.

Perhaps these pigments are controlled by multiple factors and the varying amounts produce the color differences in the cotyledon, in the flower, and in the root bulb flesh but in all probability they are superimposed upon the ground chlorophyll pigments with cumulative effect and are each controlled by individual simple factors.

In any case the cotyledons contain the same pigment as the bulb flesh and are an index of the color of the flower and of the bulb flesh. In consequence they may be used to practical advantage in the distinction of the seeds of yellow-fleshed rutabagas, which in Canada represent the rutabaga, and of yellow-fleshed turnips, by means of the very young seedling.

In Table I, the colors produced by these substances in young cotyledons are listed beside those of the anthocyanin pigments.

*The fourth group* of pigments are those to be found in the pigment layer of the seed coat or impregnated in the lignified portion of the palisade cells. More general use has been made of this group. For example, if seed coats of *Brassica arvensis* are boiled with chloral hydrate solution and examined under the microscope a red pigment bleeds from the palisade cells.

*Brassica* seeds change color on ripening and sometimes it is difficult to distinguish mature seeds. Sirks (59) proved that the red seeds commonly found mixed with the dark in certain varieties of rape were not immature seeds but typified a definite strain. Perhaps several *Brassica* seed varieties are mixtures of strains as their color would indicate because they seem to remain true to type, and seed coat color has been found to be one of the most generally reliable of the macroscopic characters of the seed for use in seed distinction.

The presence of carotinoid pigments in the cotyledon forms the basis of the following practical laboratory test.

**METHOD for the Separation of Swede Turnip and Dwarf Essex Rape Seeds by Means of the Carotinoid Pigments in the Cotyledon**

Germinate the seeds between blotters *in the dark*. It is much easier if they are spaced carefully on the blotters. Allow for seven days' growth *in the dark*, or if the cotyledons have not opened in that time, longer. Fit filter paper in a covered Petri dish and saturate it with a 75% solution of lactic acid, using 15 cc. of solution for a Petri culture dish of 100 mm. diameter. Work quickly. Clip off the cotyledons of the seven-day-old seedlings and transfer them to the soaked filter paper, carefully arranging them in rows. Let stand *in the dark* for 10 or 12 hr. It is convenient to do this work in the afternoon and study the cotyledons the next morning, checking a couple of times through the day. After 24 hr. the cotyledons begin to fade. After the cotyledons have been 10 hr. in the lactic acid, remove the chrome yellow colored cotyledons or those of the Swede turnip to one side of the Petri dish and the lemon yellow or the Dwarf Essex rape cotyledons to the other side and count to find the percentage of each in the mixture.

**IV. Descriptions and Keys for the Identification of *Brassica* Seeds and Seedlings**

*The shape of the seeds* of a variety is not always uniform, but on the other hand if the seed is placed in such a way that the raphe is higher than any other portion of the seed and the radicle faces to the left, the characteristic shape or shapes of the species may be noted and in good seed specimens these shapes make up the bulk. Thus *B. oleracea* L. has four typical seed shapes, Figs. 18, 19, and each of its varieties by contributing its own individuality to each of these four shapes gives a clue to its identification. In samples of poor quality, of course, misshapen and diseased seeds require more specific methods. It is also apparent that there is a small percentage of seeds, more especially in strains of cabbage, which do not conform, and as a consequence shape cannot be depended upon as a definite purity test for all seeds.

*The size of the seeds* is given in three dimensions, the measurements being made with calipers graduated to the sixty-fourth of an inch. The use of this small measurement serves to emphasize minute differences in the comparative size of the different seeds as well as in the different dimensions of the one seed.

PLATE I



FIG. 2. *Crystals from alcoholic ether solution.*





This measurement was multiplied by 0.4 to obtain the approximate millimeter reading. For the purposes of this record the average of 100 seeds was taken. In a sample of seed the size necessarily varies according to the conditions of growth and the screen over which the sample was cleaned. As the cleaning screen used for a variety of commercial seed is more or less constant, the average measurement of 100 seeds in different commercial samples of a variety varies surprisingly little. It must never be forgotten, however, that seeds of improved strains of a variety are sometimes exceptionally large and also that in any mixture such measurements are useless.

The surface appearance of the seed coat must also be taken into account varying as it does from finely granular to deeply pitted. Even within the species this difference is sufficient to separate two varieties far removed from each other in the series, e.g., cauliflower and marrow kale, but it is disappointing as a means of distinguishing two varieties closely related. Besides, variation within the variety is often too great.

The hilum and the raphe of the seed may often be used to distinguish species and sometimes varieties. In *B. oleracea* L. and *B. Napus* L. the raphe, which appears to be composed chiefly of a bundle of vascular elements, is definitely outside of the hilum, but in *B. Rapa* L. and *B. campestris* L. it overlaps. The hilum in *B. oleracea* L. is large, oval, and rough; in *B. Napus* L. it is large, oval, and flat; in *B. campestris* L. it is distinctly round while in *B. Rapa* L. it is very small.

The cotyledons and radicle of the seed studied when the seed coat is removed are, according to Orechova and Lapinskaja (1936 (44) ), useful in identification. There is no doubt that minor differences occur and are evident but after making a determined effort to use this method for identification it was found that these differences were too slight and irregularities in the numerous strains too frequent to make it practical. Cauliflower seeds can be identified in this way although some forms of broccoli are very similar. Brussels sprouts seeds are also typical but there are many irregularities in the cabbage seed. However, as in the case of anthocyanins, any positive characters along this line should be noted for evaluation before proceeding to another method of identification.

When all else fails the seedling plant offers a solution in the shape of the cotyledon and the first seedling leaves, in the presence or absence of an internode, and in the general appearance of the plant. Small pasteboard flats in which the seeds are planted one-half inch apart in a sterilized mixture of two parts loam and one part sand are easily handled and fit neatly into large wooden flats. Special care must be taken in the watering, and sufficient light is necessary. Check tests of closely related seeds are essential.

In the study of seedlings as means of identification of seed, much stress has been laid on the hairs of the leaves, their position, and shape. I have found these very confusing. Hairs on seedling leaves would seem to be affected by the climate, their development being responsive to stimulus perhaps of light, temperature, moisture supply, or other cause. I have found in growing seedlings both under glass and outside that under the conditions

of my experiments the presence of hairs rather than their absence, coupled with inconsistencies in their position and extent, is to be expected. Hellbo (1933 (22)) claims that some hairs on the seedling leaf of turnip are bent at a right angle and may be used as a distinguishing feature. This is quite true of some but not of all strains and doubt arises as to whether in some cases hairs accidentally bent may not be confused with those that inherit this form.

It is also claimed that the rutabaga might be identified by the presence of hairs on the front of the petiole but here again they are not present in all seedlings and they are found on some rape seedling petioles. A certain amount of caution should be used and in any case I do not believe the presence or absence of hairs sufficient to be used alone as a distinguishing feature.

Lavrova (1932 (36)) pictures very young seedlings before the seedling leaves have developed, utilizing cotyledonary shape and inherent seedling behavior of growth. As a matter of fact these two points are extremely important but in such very young seedlings the differences are so slight they are liable to be affected by physical agencies during germination.

The following descriptions apply to seedlings that have formed one or two seedling leaves. They are based on the most general features noted in at least 100 seedlings of every variety available, using the seedlings from seed tested by growing them to mature plants in the greenhouse or garden as the basis of description. Thus the descriptions are composite taken from several hundred plants whereas the illustrations are drawings from photographs of an individual plant chosen because it illustrated, perhaps emphasized, one or more representative points considered important.

For convenience of presentation the arrangement of the groups, in so far as seed character associations have allowed, is that of O. E. Schulz (1919).

The size of cotyledon, the width by the depth, measured in inches (1 in. = 25.4 mm. approx.) is the largest attained by them in the aforementioned growing tests. These were carried on without the use of artificial stimulant.

The size of the seeds was also measured in inches, the sixty-fourth of an inch being the unit used. This may be multiplied by 0.4 to give the approximate millimeter reading. Thus a seed measuring  $\frac{3.75}{64} \times \frac{3.5}{64} \times \frac{3.25}{64}$  in. is given as  $1.5 \times 1.4 \times 1.3$  mm. The measurements given, taken in three dimensions, are the average of those of 100 seeds of as many varieties as were available.

The colors mentioned refer to Ridgway's color standards (51).

1. **Brassica balearica** Persoon, C. H. Syn. pl. II. 206 (1807); Schulz, O.E., Engler's Pfl. H. 70, IV, 105. 25 (1919)

Fig. 20. Uncommon in Canada.

Seed— $2.04 \times 1.84 \times 1.64$  mm.; Natal and bone brown, hazel and chestnut brown, drab and hair brown, and wood brown. The seed is surprisingly similar to that of the common broccoli, in color except for the

absence of the deep olive gray seeds and less of the chestnut brown, in shape except for the absence of the heart-shaped seeds and the presence of a larger number of the broad shallow seeds of the kales, and in seed coat except that the regular shallow oval cavities are just a little coarser. It is generally slightly larger in size.

Cotyledon— $1.8 \times 1.3$  cm.; almost *slate gray* at opening, later becoming *glaucous blue* with reddish edges and veins; thick; translucent; one slightly larger with longer petiole; indentation wide, shallow, and angled at edges.

Seedling—sturdy; stems reddish.

Seedling leaves—dark blue green; shiny; thick; glabrous; veins tinted red at base; ovate, acuminate at base with or without particles down stem; margin irregularly incised; surface undulate.

2. ***Brassica macrocarpa*** Gussoni, G., Ind. Sem. Hort. Reg. Boccadifalc 3 (1824-5); Schulz, l.c. 26

Fig. 21. Uncommon in Canada.

Seed— $2.0 \times 1.8 \times 1.5$  mm.; hair brown, Natal and bone brown; resembling thousand-headed kale in shape but lacking the broad shallow seed of the kales; the seed coat has a regular, fine, medium deep stippling.

Cotyledon— $0.79 \times 0.95$  cm.; *dark green* with lighter colored veins tinted purplish-red at base; shiny; thick; heart-shaped and continuous with the petiole in a graceful sweeping curve; *indentation* narrow, deep, and arcuate; distinguished from *B. oleracea* L. by its color.

Seedling—fine; petiole green, tinted purplish-red.

Seedling leaves—green covered with a white glaucous coat; thin; glabrous; oblong to ovate, acuminate at base; margin irregularly but somewhat biserrately incised; surface undulate.

3. ***Brassica oleracea*** L. Spec. Pl. Ed. i.II.667 (1753)

Distinctive features of the species:

- (i) Seed color gray and brown predominating (see seed key); seed size varying (see text), and seed shape typical (see Figs. 18 and 19). The raphe is always outside of the hilum, which is large, oval, and rough. The micropyle is above the usually raised tip of the radicle ridge.
- (ii) Radial section of the seed coat (Fig. 4).
- (iii) Purple anthocyanins are common to the colored strains of the species but are lacking in the white strains. Table I.
- (iv) Seedlings have a glaucous coat, varying slightly in color and intensity according to variety but typical and easily recognized.

A. *Brassica oleracea* L. var. *ramosa* (DC). Alefeld., Landw. Fl. 234 (1866); Schulz, l.c. 30

*B. arborea* Steudel, E.G., Nom. Bot. ed. i. 116 (1821)

*B. oleracea* L. *B. acephala* DC. subvar. *ramosa* DeCandolle, O.P., Syst. Nat. II. 583 (1821) et. Prodr. 1. 243 (1824)

Bush kales, branching kales.

I. Flanders kale. Fig. 22. Not common in Canada.

Seed— $1.8 \times 1.76 \times 1.6$  mm.; olive gray, olive and clove brown, fuscous; majority of seeds are quite as wide as high but a few of the tall type of seed as found in thousand-headed kale are present, seeds often come to rest with the hilum and the micropyle visible; seed coat has an extremely shallow primary net and a conspicuous secondary net, and is generally fine with buff-colored wax in patches on the surface.

Cotyledon— $1.1 \times 0.79$  cm.; often three cotyledons of deep dull yellow-green; shiny; thick; triangular; *indentation* very broad, shallow, and rounded.

Seedling—strong; slow growing; short; stem green; hypocotyl tinged with red.

Seedling leaves—slightly glaucous; oval, truncate tip and base; coarsely, irregularly, and deeply serrate with four serrations on each side.

II. Thousand-headed kale. Fig. 23. Extensively grown in Canada.

Seed—Fig. 18;  $2.04 \times 1.8 \times 1.64$  mm.; olive gray, olive and clove brown; rough, viewed from above elongated and slightly *wedge-shaped*; very heavily coated with *buff-colored wax* some of which chips off; primary net in the seed coat, angled, its walls shallow, narrow, and perpendicular; secondary net appears in horizontal rows across seed; seed coat very thick.

Cotyledon—very small in some strains but may reach  $1.27 \times 0.95$  cm.; chromium green; thick but thinner than broccoli; one larger and kidney-shaped, other inclined to be heart-shaped but both have flared bases; closely resembles sprouting broccoli in shape but more like cabbage in texture and in not bending at right angles to the petiole; *indentation* medium broad, deep, and rounded.

Seedling—irregular; sturdy; tall; stem thick sometimes purplish at base; slower growing than broccoli. Table I.

Seedling leaves—*glaucous gray above and below* with trace of purple at base; thick with veins prominent although not so deeply sunken as in marrow kale; glabrous; obovate to cuneate; shallowly sometimes sharply serrate and biserrate.

**B. *Brassica oleracea* L. var. *acephala* DC. l.c. 583; Schulz l.c. 30**

Leaf kales—grown only to a limited extent in Canada.

**I. Kitchen kales.** They are variously classified according to color, habit of growth, and the degree of laciniation of the leaf.

**a. Tall green curled, or Scotch kale; intermediate moss curled kale; dwarf green curled or Labrador kale. Fig. 24.**

Seed— $1.9 \times 1.72 \times 1.64$  mm.; *clay color* with seeds of *tawny olive* conspicuously present; primary net extremely shallow on the seed coat.

Cotyledon— $1.43 \times 1.1$  cm.; light bice green later becoming darker; dull; deep central vein; indentation circular and deep, broader in one than the other.

Seedling—fine; slender; tall green kale is a taller seedling and has a narrower indentation in the cotyledon; *stem petiole and median vein* a characteristic *apple green*. Table I.

Seedling leaves—bice green slightly blue glaucous above and slightly gray glaucous beneath; thin; dull; upright; usually glabrous but may have setose hairs on the edges; appearing early and early showing the undulate pinnasecti character of the leaf.

**b. Asparagus kale or Jerusalem green curled kale. Fig. 25 b.** Grown in Canada but not common. Asparagus and ragged Jack kales have been placed by many writers along with the winter and oil rapes, *B. Napus* L. While these seeds resemble *B. Napus* L., Fig. 6, in not having a celled outer epidermal row in radial section of the seed coat, they have the tumbler cells with narrow straight-sided walls of uniform height, flattened above and below, so typical of *B. oleracea* L. varieties, Fig. 4. They are without doubt intermediate forms.

Seeds— $1.76 \times 1.7 \times 1.6$  mm.; shaped like the rape they normally rest with the hilum above; the hilum, raphe, and micropyle are also similar to those of the rape in size, shape, and placement. However, the mucilage that often covers the hilum and that turns brown on old seeds, the blue gray color of the seed, and the large rectangular meshed waxed net in the seed coat are oleraceous characters.

Cotyledons— $9.5 \times 6.3$  mm.; green; not glaucous; thick; indentation small, rectangular.

Seedling—purple hypocotyl, Table I, slow growing; slender; tall; medium green; stem dark brownish-green.

Seedling leaves—Rinneman's green; not glaucous; thin; may have setose hairs on under veins, edges, and petioles; broadly ovate with truncate tip so that it is quite as broad near to the tip as at the center, but as the leaf gets older it becomes almost round; unevenly dentately serrate.

- c. Variegated kale. Fig. 28 c. A garnishing kale, grown more extensively in Canada than some other strains. Sometimes mixed with ribbon kale.

Seed— $1.86 \times 1.67 \times 1.59$  mm.; shapes more nearly resemble those of collards but seeds are more angular and have no raised raphe; shallow forms predominate; net with fine, shallow, squared mesh, of uniform size, and without pattern in the seed coat.

Cotyledons—similar to those of the green kales.

Seedling—opens like that of marrow kale.

Seedling leaves—similar to those of the green kales except for the deep red and blue *anthocyanin* coloring in the lamina. Also they are more ovate and not so deeply incised.

- d. Blue curled Siberian kale. A dwarf kale; quite commonly grown.

Seed— $1.76 \times 1.68 \times 1.6$  mm.; deep dark mouse gray; somewhat spherical; primary net is distinct but so shallow as to approach in appearance that of *B. Napus* L.

Cotyledons—small; glaucous; asphodel green; dull; thick; heavy; dark purplish-red median vein; indentation broadly triangular.

Seedling—slender; stem and petiole purplish-red.

Seedling leaves—blue glaucous; bristly setose on upper lamina, edges, and petioles; edges deeply cut.

- e. Collards. Fig. 26. Plant cut for food in the rosette stage but if left will form a head. Not common in Canada.

Seed— $1.84 \times 1.72 \times 1.2$  mm.; Fig. 18; viewed from above a rounded oval; primary net shallow; small; fairly regular; rectangular.

Cotyledon— $9.5 \times 4.76$  mm.; lamina ribbon down stem; deep dull yellow-green; thick; when young sharply angled with the petiole but later straightening; indentation arcuate.

Seedling—gracefully fine; stem greenish-white or purplish-red.

Seedling leaves—shiny; thick; glabrous; veins broad and white; oval or broadly elliptical with obtuse tip and acuminate base; sharply and shallowly serrate.

- f. White ribbon kale. Fig. 27 a. Mature leaves cut in ribbons but plant forms a head in the fall. Often grown for ornamental purposes.

Seed— $1.96 \times 1.88 \times 1.64$  mm.; mouse gray; heavily coated with light brown mucilage; primary net very shallow in vertical rows down the rectangular side of the seed; viewed from above ovate.

Cotyledon— $9.5 \times 7.9$  mm.; white green; thin; dull; one erect the other forming an angle with the petiole; lamina ribbon for short distance down stem; indentation narrow and deep.

Seedling—small; opening like marrow kale; petiole with red tinge.

Seedling leaves—*white green*; glabrous; veins deep, sometimes purplish; oval with tip and base slightly acuminate but so deeply cut as to give the impression of lobing.

II. Borecoles and kales. Used for the most part as stock food.

a. Sheep kale, kale rape. Grown in Canada in limited quantity.

Seed— $1.48 \times 1.44 \times 1.44$  mm.; regularly *spherical*; uniformly deep mouse gray.

Cotyledon— $1.27 \times 1.59$  mm.; dark green; purplish petiole; shallow, broad, rounded indentation.

Seedling—very small; oleraceous. Table I.

Seedling leaves—deep set veins in a smooth surface; hirsute on edges and on under lamina; purplish tint in veins, stem, pedicel, and leaf edge.

b. Cottagers kale. Fig. 27b. Uncommon in Canada.

Seed— $1.96 \times 1.76 \times 1.6$  mm.; all shades of drab and cinnamon drab; coated with bronze mucilage; viewed from above, rectangular and from the side, the lower left corner is cut away; net small-meshed and shallow, *juncea*-like.

Cotyledons— $1.1 \times 0.8 \times 1.1$  cm.; one being narrower than the other; broadly heart-shaped; light green; thick median vein; indentation narrow and triangular.

Seedling—tall and slender; purplish stem.

Seedling leaves—slightly glaucous; shiny; glabrous, orbicular to peltate; more or less dentately serrate or biserrate.

c. Milan kale. Fig. 28 b. Uncommon. Fleshy shoots are cut in the spring and later a small head forms at the top of the plant.

Seed— $1.8 \times 1.8 \times 1.8$  mm.; tending to *cubical*; shades of drab and olive brown; a net minutely shallow, of small rounded mesh.

Cotyledons— $7.9 \times 9.5$  mm.; broadly heart-shaped, attenuate at base and continuing down stem; indentation deep, angular.

Seedling—tall; slender; graceful.

Seedling leaves—glabrous not glaucous; unsymmetrical; oval; acute tip and cuneate base; secondary leaf with a more truncate tip and more biserrately cut.

d. Marrow kale. Fig. 29. A thick stemmed variety extensively grown.

Seed— $2.7 \times 1.96 \times 2.2$  mm.; Fig. 18; dark olive buff, mouse and olive gray, olive and clove brown; dotted with white mucilage; viewed from above wedge-shaped to oval and round; seed coat very *thick*; primary net deep; mesh small with slanting walls.



Cotyledon— $1.1 \times 1.27$  cm.; medium dark green; thin; veins white or purple; *cotyledon completely circular* if the curved irregular indentation and the narrowing of the lamina down the stem are ignored.

Seedling—on opening, the upper larger cotyledon has its edges loosely rolled inward to form a semicircle wholly imbricated within the lower curved smaller one. As the inner elongates the outer remains slightly curved. They then open by separating on one side only; slender; tall. Table I. Stem and under veins white with purple, or green with bronze.

Seedling leaves—light bluish-green; not glaucous, unsymmetrically and deeply serrate or triserrate.

- e. Ragged Jack kale\*. Fig. 25 a. A very common impurity in samples of seed received from southern Europe.

Seed— $1.88 \times 1.68 \times 1.6$  mm.; deep mouse gray; almost spherical.

Cotyledons— $6.35 \times 7.9$  mm.; very dark green; the upper when young at right angles to an erect petiole; hypocotyl on germinating curling in an almost complete circle.

Seedling—sturdy. Table I.

Seedling leaves—glabrous; *three- to four-parted with each part again incised*.

C. *Brassica oleracea* L. var. *bullata* DC. subvar. *palmifolia* DC. l.c. 584

Palm tree kale. Fig. 28 a. Uncommon, often used for ornamental purposes but one variety in South America is used for food.

Seed— $2 \times 1.6 \times 1.6$  mm.; viewed from above round, from the side resembling the rounded and taller forms of savoy cabbage as pictured in Fig. 19.

Cotyledon— $9.5 \times 7.9$  mm.; one erect, heart-shaped, other angled with petiole, much broader; indentation shallow; curved.

Seedling—*bice green stem and petiole* (cf. green kales).

Seedling leaves—somewhat glaucous; shiny; wrinkled; slightly setose on under lamina; obovate sometimes with cuneate base; the primary having a blunt tip; shallowly and crenately dentate.

D. *Brassica oleracea* L. var. *sabauda* (L.) Martens in Mart. et Kemmler, Fl. Wurttemb. ed. 2. 35 (1865); Hegi, R. Illustr. Fl. Mitt. Eur. IV. i. 246 (1917)

*B. oleracea* L. var. *bullata* DC. subvar. *sabauda* (L.) DC.; Schulz l.c. 31

*B. sabauda* Dod. Pempt. 624 (1583) emended (1616); Schulz l.c. 31

Savoy cabbage, Fig. 30. Common vegetable.

\* See *asparagus kale*.

Seed— $1.96 \times 1.8 \times 1.64$  mm.; Fig. 19; vinaceous drab, pecan brown, Rood's brown, fuscous and fuscous black; viewed from above rounded; seed coat thin; smooth; net very shallow.

Cotyledon— $9.5 \times 5.9$  mm.; the large cotyledon turned back at right angles to its tall petiole overlapping to about one-half of the small cotyledon turned back at right angles to its short petiole; puffed in center as a *balloon top*, Fig. 30; chromium green; shiny; thin; sides rounded; lamina continuing down stem; indentation irregular.

Seedling—tall; slender; graceful; lacking the stiffness of the ordinary cabbage seedling; stem light bice green.

Seedling leaves—dark greenish glaucous; smooth; dull; glabrous; veins sunken; oblanceolate to oval; edges of lamina at center of base tending to curve inwards and form a funnel.

E. *Brassica oleracea* L. var. *gemmifera* (DC.) Zenker. Fl. Theuringen XV. 2 (1824); Bailey l.c. 226

*B. oleracea* L. var. *bullata* DC. subvar. *gemmifera* (L.) DC.; Schulz 31

*B. gemmifera* Léveillé Monde. Plant XII. 24 N.V. (1910)

Brussels sprouts. Fig. 31. Common vegetable.

Seed— $1.96 \times 1.76 \times 1.52$  mm.; Fig. 18; Saccardo's umber and sepia, russet and hazel; viewed from above rounded; seed coat thin, smooth. Figs. 3, 4.

Cotyledon— $1.43 \times 1.1$  cm.; chromium green becoming lighter; smooth; shiny; on germination the hypocotyl almost perpendicular and in line with the petiole and the two cotyledons also standing *perpendicularly with ventral faces together*; larger cotyledon with broader indentation and more acuminate base.

Seedling—short; stout; hypocotyl, under veins, petiole, and stem green, with bronze or purple, according to variety.

Seedling leaves—Rinneman's green; smooth; glossy; glabrous; primary leaf oval, practically *entire* on a short *erect* petiole. Table I.

F. *Brassica oleracea* L. var. *capitata* L. Spec. Pl. 667, ed. I, II (1753)

Cabbage. Fig. 32. Common vegetable.

Seed— $2 \times 1.84 \times 1.68$  mm.; improved varieties much larger; Fig. 19; testaceous, cocoa brown, walnut brown, burnt umber, deep brownish drab, fuscous, and a few deep olive gray; noticeable ridge in upper left side caused by an enlarged radicle tip emerging from between the cotyledons in the seed halfway up the side; irregular, more often wider than deep; primary net, with narrow, straight-sided walls and of large squared mesh, in regular rows.

Cotyledon— $14.2 \times 7.9$  mm.; in germination the smaller cotyledon opening immediately on being pushed above the soil, the larger one becoming conduplicate, ventral side within, for some time before moving slightly to one side, bending back, and opening away from the other on the opposite side; chromium green; shiny; thin; kidney-shaped; heart-shaped in some strains; puffed at center as in savoy cabbage; indentation arcuate.

Seedling—very sturdy; stem and petiole thick and white with a possible trace of purple. Table I.

Seedling leaves—*funnel-shaped at base*; smooth; dull; pronounced deep greenish glaucous; on sprouting, the primary leaf, conduplicate with ventral side within, is extended at right angles to the stem by the growing petiole, which later straightens to a vertical position and holds the *stiff primary leaf turgidly erect*; broadly oblanceolate to oval with obtuse-angled tip but varying according to strain; shallowly, evenly, and finely biserrate.

G. *Brassica oleracea* L. var. *botrytis* L.

I. *Brassica oleracea* L. var. *botrytis* L. subvar. *cymosa* Duchesne, Lamarck, Encycl. I. 745 (1783); (*asparagoides* DC. l.c. 586 (1821)

*B. oleracea* L. var. *italica* Plenck, Plant Medic. VI, 29.t.534 (1794)

*B. asparagoides* Calwer, Deutochl. Feld. u. Gartengew. 183. t. 28 (1852) Bailey l.c. 227.

Broccoli. Fig. 34. As a vegetable in greatly increasing demand.

1. Types with small heads forming in the axils of the leaves: Italian green sprouting, Calabrese, Propogena.
2. Types resembling cauliflower only maturing later: Walcheren, Early Roscoff.

Seed— $1.76 \times 1.72 \times 1.6$  mm.; Fig. 19; hazel and chestnut brown, Natal and bone brown, few deep olive gray and wood brown; viewed from above rectangular and from the side an elongated oval flattened on upper right corner; seed coat resembling cauliflower with the exception of the deeper net and the mesh four times as large.

Cotyledon—deep dull yellow-green; blue glaucous; shiny; thin; although thicker than thousand-headed kale; in Type 2, resembling that of cauliflower, though slightly less angled, but in Type 1, *resembling those of thousand-headed kale*; indentation broad, shallow, and more rounded than that of the kale.

Seedling—tall; slender at first; *slow growing*; stem greenish-white tinged at the base with purple and bronze, Table I.

Seedling leaves—*deep greenish glaucous*; glabrous; smooth; primary leaf, when sprouting, slender, conduplicate, ventral side within, and inverted by a graceful curve in the petiole; ovate with obtuse or acute tip; unsymmetrical; crenately biserrate.

II. *Brassica oleracea* var. *botrytis* L. subvar. *cauliflora* (Gars.) DC. l.c. 586; Schulz. l.c. 33

*B. cauliflora* Garsault, Explic. Sept. Cents. dix-neuf pl. 123 (1765)

*B. botrytis* Mill. Gard. Dict. Ed. 8 (1768); Bailey l.c. 229

Cauliflower. Fig. 35.

Seed— $2.16 \times 1.72 \times 1.68$  mm.; Fig. 19; drab and hair brown, Natal and bone brown; viewed from above rounded, from the side elongated; coat *fine*; *smooth*; thin; with an exceptionally shallow net, square-meshed.

Cotyledon—*blue glaucous leaf green*; thick; translucent; triangular in shape with apex toward the petiole; about 1.5 mm. from this apex bending at a sharp right angle to the perpendicular stem (Fig. 35 *b*) to form a *flat symmetrical table, rectangular*  $2.2 \times 1.58$  cm., except for the clearly defined angular incisions in each of the four sides (Fig. 35 *c*).

Seedling—sturdy; slow growing; stem, petiole, hypocotyl, and under veins purplish or greenish-white according to variety; petioles elongate greatly. Table I.

Seedling leaves—erect; *deeply greenish glaucous*; dull; glabrous; oblanceolate; finely and very shallowly serrate.

H. *Brassica oleracea* L. var. *gongylodes* L. Spec. Pl. 667 (1753); Kerner, oekon, pfl. IV. 6 t. 344 (1791); Schulz l.c. 32

*B. oleracea* L. var. *caulorapa* DC. *α communis* DC. l.c. II. 586

*B. caulorapa* Pasq. Cat. Ort. Bot. Napoli 17 (1867); Bailey l.c. 232

Kohlrabi. Fig. 33. Common vegetable.

Seed— $2.2 \times 1.92 \times 1.8$  mm.; Fig. 19; but varying from 1.78 mm. to 2.18; sorghum brown, Hay's brown, and light seal brown, brownish drab, dusky drab, and blackish-brown with an occasional seed of Morocco red and of claret brown; primary net extremely shallow, honeycomb-meshed, with narrow slanting walls often capped with blue-gray mucilage.

Cotyledon— $1.58 \times 9.5$  mm.; chromium green; shiny; glaucous; short lamina ribbon down stem; indentation *broader in one than in other*.

Seedling—*very slender, tall, and graceful*; stem, hypocotyl, and petiole showing traces of vinaceous purple, Table I.

Seedling leaves—dark bluish glaucous; thick, only central vein showing; oblong lanceolate; stands erect on a *tall graceful petiole*; four serrations on one side, three on the other; end lobe elongated.

4. ***Brassica alboglabra*** Bailey, Gent. Herb. i. 79. Fig. 29 (1922)

*Brassica oleracea* (L.) Burkill, Gard. Bul. Str. Settlement V. 3-6 (1930)

Chinese kale, guy lon, sawi hijau tuah. Fig. 36. Seed and green plants in season commonly sold by Chinese merchants. The large bundles of stout short stems are conspicuous because of the large white flowers often present. Used for soups, salads, and chop suey.

Seed— $2.12 \times 1.92 \times 1.72$  mm.; deep, dark, and blackish mouse gray; viewed from above circular, from the side shield-shaped rounded at the top; net shallow, of medium large square mesh with narrow walls; sparsely spotted with gray mucilage.

Cotyledon— $1.43$  cm.  $\times$   $9.5$  mm.; turtle green; dull; thick.

Seedling—conspicuously oleraceous; stem tender, white with light purplish tint. Table I.

Seedling leaves—*olivine green*; decidedly glaucous; setose; broadly obovate to round with truncate tip; veins white and large; unsymmetrical; sharply biserrate.

5. ***Brassica Robertiana*** Gay, Ann. Sc. Nat. i. VII. 416 (1826)

*Brassica Montana* Pourret, Chlor. Narb. n. 180. m. Mem. Acad. Toul. i. III. 308 (1788); Schulz l.c. 33

Fig. 37. Uncommon.

Seed— $1.88 \times 1.8 \times 1.48$  mm.; russet and hazel, Saccardo's umber and sepia; almost spherical somewhat resembling seeds of cauliflower in shape but lacking its graceful spherical curves; seed coat thin; primary net of squared mesh, fine and irregular.

Cotyledon— $1.1 \times 1.1$  cm.; deep, slate green when young, later becoming lighter always blended with bright, purplish red; dull; waxy; heart-shaped with a slight angle at the sides; lamina continuing down stem for a short distance; indentation the shape of the two end lobes of the cotyledon inverted.

Seedling—large; sturdy; stem and veins purplish-red; petioles long and glaucous gray.

Seedling leaves—dark greenish glaucous; glabrous; oval to ovate later elongating slightly; unevenly bidentate; unsymmetrical.

6. *Brassica insularis* Moris, Fl. Sard. I. t. XI. 168 (1837)

Fig. 38 a. Not commonly grown.

Seed— $1.8 \times 2 \times 1.36$  mm.; Chaetura drab, mars brown, russet brown, heavily spotted with brownish gray mucilage; viewed from above rectangular, from the side triangular with the top and right sides forming a right angle while the third side is arcuate; higher than broad; medium fine net, irregular.

Cotyledons— $1.1$  cm.  $\times$   $7.9$  mm.; *asphodel green*; medium thin; base red; sides rounded; indentation broad, very shallow and arcuate.

Seedling—large; stems red, petioles elongated.

Seedling leaves—*deep greenish glaucous*; glabrous; large; broadly oval to obovate; undulate, sharply dentate.

7. *Brassica cretica* Lam. Encycl. 747 (1783); Schulz l.c. 36.

Fig. 39. Uncommon in Canada.

Seed—light grayish-brown; viewed from above round, from the side oval; primary net fine and indistinct in rows in the seed coat.

Cotyledons— $1.4 \times 1.1$  cm.; deep slate gray; dull; waxy; thick; veins purplish-red; indentation narrow; deep and curved.

Seedling—large; *glaucous green*; stems purplish-red.

Seedling leaves—glabrous; dark green; blue glaucous; dull; veins purplish-red, sunken; broadly oval, more or less truncate at tip and base; quickly enlarging; lamina rough; repand; shallowly and crenately biserrate.

8. *Brassica incana* Tenore, Prodr. Fl. Napol I. i. (1811–15) XXXIX, et IV, Syll.t. 165 (1830); Schulz l.c. 37

Fig. 38 b. Uncommon in Canada.

Seeds—large; spherical but slightly elongated; cocoa brown, walnut brown, burnt umber, deep brownish-drab; primary net clear-cut, medium fine and irregular, secondary net fine.

Cotyledon— $1.58 \times 1.1$  cm.; *almost slate gray* remaining dark; dull; thick; translucent; waxy; veins reddish; heart-shaped; indentation shallow and narrow.

Seedling—oleraceous in appearance; *stem hoary gray*.

Seedling leaves—very dark green; glaucous blue above, hoary gray beneath; dull; broadly oval with truncate tip; shallowly and irregularly biserrate.

9. *Brassica pekinensis* Ruprecht, Fl. Ingr. I. 96 in textu (1860); Bailey l.c. 250

*B. cernua* (Thunb.) Forbes & Hemsley, J. Linn. Soc. XXIII, 47 (1886); Schulz l.c. 59

*B. campestris* L. sub. sp. *chinensis* L. var. *pekinensis* Burkill.

Pe-tsai, chu toy, celery cabbage. Pe-tsai is a general term but is often commercially associated with the open-headed type of plant. Its use as a vegetable is gradually increasing.

General characters of the species:

1. Seed— $1.9 \times 1.76 \times 1.56$  mm. with a wide range of size; vinaceous; viewed from above wedge-shaped, thickest at radicle, from the side circular with an elbow one-third from the top on the right side with the lower right side cut away; net shallow, round-meshed and small; raphe, round and quite the largest of the Brassicas, on the base of the hilum, both enclosed by a second ring.
2. Distinguished from *B. Napus* L. by the brownish-yellow seed coat, sloughed off on germination, that contrasts with the very dark one of the rapes—a color difference not apparent in the dry seed.
3. Seedling stem succulent; white with purple tint; Table I.
4. Shape of seedling leaves is an index to the shape of the mature head.

The following six varieties are grown:

A. Chihili or improved Peking. Fig. 41.

Head large, hard, projectile-shaped.

Seed— $1.8 \times 1.66 \times 1.5$  mm.; light and dark vinaceous drab and dark grayish-brown, vinaceous rufus and liver brown, and a few neutral gray; more elongated than other varieties; *oval bodies* in pigment layer of the seed coat; net small and very shallow; secondary net visible.

Cotyledons— $1.43 \times 1.27$  cm.; dull apple green becoming bice green; indentation narrow, deep, and angular.

Seedling—tall; slender; stem purplish.

Seedling leaves—*rapidly elongating*; long hairs on edges, shorter on surface, under veins and petioles; veins prominent, white, broad at base; *oblanceolate* with lamina ribbon down stem; finely and sharply biserrate.

B. Wong bok. Fig. 40.

Head half-long; large; tightly wrapped.

Seeds— $1.8 \times 1.76 \times 1.7$  mm.; brownish-drab and dusky drab, hazel and chestnut brown, cinnamon rufus; elbow low and prominent; coarsest primary net of group; secondary net distinct, fine.

Cotyledons— $1.44 \times 1.1$  cm.; lamina ribbon down stem; turtle green; indentation shallow, arcuate.

Seedling—short; stout; stem and petiole thick.

Seedling leaves—decidedly setose; *round* when young later broadly obovate, acuminate at base; veins prominent; coarsely and shallowly dentate and bidentate.

C. Chefoo. Fig. 42.

Head—large; round; solid.

Seed— $1.9 \times 1.79 \times 1.55$  mm.; dark and light, vinaceous drab, dark grayish-brown, and neutral gray; seed angular; very shallow and flattened primary net.

Cotyledons— $1.76 \times 1.6$  cm.; dull; medium thick; large; veins prominent; indentation broad, circular.

Seedling—large.

Seedling leaves—densely setose; thin; dull; slightly wrinkled; obovate; becoming more acuminate; unsymmetrical, shallowly dentate.

D. Market Pride. Fig. 43.

Head—large, tender, loosely folded like a cabbage.

Seed— $1.87 \times 1.8 \times 1.7$  mm.; brownish- and dusky drab, Hay's russet, liver brown and deep mouse gray; prominent elbow; primary net slightly smaller than in wong bok, secondary net distinct.

Cotyledons— $1.43 \times 1.27$  cm.; thin; light bice green; appearing almost rectangular; indentation shallow; angular.

Seedling—*stalky*.

Seedling leaves—sparsely setose; wrinkled; veins broad, white; round becoming oval acuminate at base; almost entire but coarsely and shallowly dentate and bidentate.

E. Shantung.

Head—large; loose and open.

Seed— $1.79 \times 1.67 \times 1.44$  mm.; dark and light vinaceous drab; dark grayish-brown, few vinaceous rufus and neutral gray; side view rounded; primary net like chihili but deeper.

Cotyledons— $1.27$  cm.  $\times 7.9$  mm.; veins prominent for short distances; indentation broad and angular.

Seedling—medium coarse; short petioles.

Seedling leaves—purplish glaucous on under side; dull; not wrinkled; veins prominent; setose on edges; spatulate to obovate with acuminate base; almost entire but shallowly serrate.



## F. Chokurei.

Head—solid for over three-quarters of its length but from there the leaves flare like lettuce.

Seed— $1.8 \times 1.8 \times 1.56$  mm.; resembling wong bok in color but has very fine primary net; conspicuous micropyle and high elbow.

Cotyledons— $1.59 \times 1.8$  cm.; lamina angled at base of side; light bice green; indentation angular.

Seedling—low and medium stout; stem and veins white with mauve tint.

Seedling leaves—shiny; smooth; densely hirsute on edges and under veins but sparsely on lamina; elliptical slightly acuminate at base; long petioles; sharply denticulate.

10. *Brassica Napus* L. Spec. Pl. ed. i. II. 666. n. 3 (1753)

A. *Brassica Napus* L. var. *arvensis* (Lam.) Thellung f. *biennis* (Schubl. et Mart.) Thellung, Hegi. l.c. 254

B. *Napus* L. var. *biennis* (Schubl. et Mart.) Reichb. Deutschl. Fl. I. 107. t. 93 (1837–38); Schulz l.c. 44

Giant, broad-leaved, or dwarf rapes. Figs. 5, 6. Grown in Canada for pasture, silage, and cover crop. Produces seed the second year but very liable to winter killing.

Dwarf Essex rape. Figs. 45 and 47 a. Extensively grown.

Seed— $1.84 \times 1.76 \times 1.76$  mm. *with range to 2.12 mm.*; naturally rests with hilum visible; shiny; purplish-gray, dusky and dark neutral gray with some claret brown; viewed from above, smooth, flattened, broadly oval with flattened radicle side; black micropyle; hilum large and broadly oval with cells distinct from seed coat netting; raphe small, outside the clear-cut hilum line but connected with it by two threads of mucilage that circle the central scar in the hilum; from the side, shield-shaped with a wedge-shaped slice cut from the right top; seed coat reticulated; primary net shallow, elongated longitudinally; secondary net broadly rectangular, regular.

Cotyledon— $9.5$  mm.  $\times 1.43$  cm.; deep dull yellow-green; dull; thick; *translucent*; angled with petioles, one of which is longer than the other; indentation broad, but narrowly arcuate. Base also narrowly and shallowly arcuate on each side of petiole; lamina ribbon down petiole.

Seedling—strong; internode; no anthocyanins; Table I.

Seedling leaves—turtle green; blue pruinose; smooth; sparsely hirsute on edges, veins, and lamina; hairs on lamina set in small cushionlike projections; shield-shaped base with end lobe as a flattened dome above the shield; four lobes on each side that may be serrations, bi- or triserrations and basal one often only a fragment down petiole; secondary leaf more rectangular.

*B. Brassica Napus* L. var. *arvensis* (Lam.) Thellung f. *annua* (Schubl. et Mart.) Thellung, Hegi l.c. 254

- I. Colza rape, winter rape, oil seed winter common. Figs. 44 and 47 *b, c*. In warm countries winter rape is sown in the fall, and plants, after supplying winter pasture, produce seed in early summer.

The term "Colza" at one time was synonymous with *B. campestris* (L.), probably because these seeds were commonly used in oil production. Now, according to Passerina (1935 (47)) the term is attributed to salad oils and the seeds of the *Cruciferae* that produce them whether they be *Brassica*, *Sinapis*, *Eruca*, or *Camelina*.

Seed— $1.92 \times 1.84 \times 1.8$  mm.; dark or blackish mouse gray; spherical; normally rests with the large flattened oval hilum visible; raphe outside but from it three strands of mucilage fan over the hilum; top of seed not so smoothly flattened as biennial rape, often depressed at micropyle; palisade cells more nearly the same height than in biennial form and primary net is shallow; mesh squared or elongated longitudinally; seed coat netting continuous inside the outer hilum ridge.

Cotyledon— $1.59$  cm.  $\times$   $7.9$  mm.; deep dull yellow-green; translucent; kidney-shaped; bent at a slight angle to the stiff petiole slightly back from its junction with it; two petioles almost the same length; base broadly arcuate on each side of petiole so that in shape cotyledon appears as two circles touching at midvein; indentation broadly arcuate. This typical cotyledon is not so marked in annual oil seed rape of South American origin grown in Canada.

Seedling—fine despite large cotyledon; stems white; internode.

Seedling leaves—typically bright Rinneman's green; blue pruinose; very sparsely setose on edges and veins; veins slightly sunken; either three or four lobes on each side or three on one and four on the other, each serrate or bi- or triserrate; no segments down stem; dome of leaf often elongated.

## II. Small-seeded oil rape.

Seed— $1.75 \times 1.75 \times 1.5$  mm.; uniform blackish mouse gray; smooth; viewed from above, oval, flattened at radicle; from the side, broad, shield-shaped, very slightly curved on top and of equal width and height; rounded at base and only slightly flattened at upper right corner; primary net fine with very shallow round mesh.

Cotyledons—large, resembling Colza rape.

Seedling leaves—Rinneman's green; thick; large; smooth, very sparsely setose on edges, veins, and under petiole; primary leaf square; secondary square to oblong; large with an extremely shallow broad dome; lamina down stem in shallow wings; unevenly biserrate.

- C. *Brassica Napus* L. var. *quadriwalvis* Hook. f. & Thoms. J. Proc. Linn. Soc. Bot. V. 170 (1861); Schulz. l.c. 42

Fig. 46. Uncommon.

Seed— $1.8 \times 1.8 \times 1.6$  mm.; viewed from above round, from the side an elongated oval; irregular; net pentagonal-meshed and medium deep.

Cotyledons— $1.1$  cm.  $\times$   $7.9$  mm.; turtle green; medium veins marked for short distances; indentation deep and narrow.

Seedling—tall; bright green stem; purple nodes; internode.

Seedling leaves—glabrous or very sparsely setose; shiny; wrinkled; obovate with acuminate base; dentately serrate.

11. ***Brassica napobrassica*** (L.) Mill. Gard. Dict. ed. 8 (1768) Bailey l.c. 237.  
*B. Napus* var. *napobrassica* (L.) Reichb. Mossler's Handb. Gew. 3. ed. II. 1220 (1833)

Swede turnip or rutabaga. Figs. 47 *d*, 48. Cultivated for food. Each of the many strains of Swede turnip according to size and color of seed, or to seedling size, edge of leaf, and intensity of blue glaucous color may be arranged in a series from those that resemble the rapes on the one hand to those close to the turnip on the other. Yellow Williamsburger, Improved Lord Derby, and Stirling Castle are more rapelike, while Improved Lothian and Ewing's Invictus appear to resemble certain turnip strains.

a. Yellow fleshed strains.

Seed— $1.76 \times 1.72 \times 1.68$  mm.; with a range of  $1.7$  to  $1.96$  mm. dusky with blackish-brown, auburn, deep and dark neutral gray with traces of red brown; more or less cubical; raphe outside of the oval hilum; radicle ridge present; micropyle broad and black.

Cotyledon—Rinneman's green; very slightly glaucous; thicker and more translucent than turnip; veins visible; size and shape variable; two cotyledons are equal and symmetrical. Lycopinoids in cotyledons of seven-day seedling distinguishes it; Tables I and II.

Seedling—strong; quick growing; *no internode*; hairs on inner face of petiole.

Seedling leaves—becoming more glaucous with age; more or less setose; dull; wrinkled to smooth; broadly oval to round; *shallowly* and sharply triserrate.

b. White fleshed Swede turnip or rutabaga.

Uncommon in Canada.

Seed and seedling characters similar to yellow-fleshed strains with the exception that no color tests apply. Exceptions to the presence of hairs on the inner side of the petiole, sometimes used as a distinctive feature of the Swede turnip, are to be found in both turnips and Swedes. The presence of an internode (Gorman and Lafferty, 1931 (18); Robson, 1934 (53)) was also noted in some Swede turnip seedlings. Thus these points are only indicative and the only positive point of distinction between yellow-fleshed Swedes and dwarf Essex rape in the seven-day seedling stage lies in the distinctive color of the young seedlings grown in the dark. Identification of white-fleshed Swede turnips has to be based on the well developed seedling.

12. **Brassica Rapa** L. Spec. Pl. 666, Figs. 124 and 125 (1753)

*B. campestris* L. var. *Rapa* (L.) Babington, Man. Brit. Bot. ed. 3. 24 (1843)

Tuberous rooted forms.

A. *Brassica Rapa* L. var. *rapifera* Metz. Syst. Besch. cult. Kohllart. 50 (1833)

*B. Rapa* L. var. *Rapa* Thellung, Hegi. l.c. 261

*B. Rapa* L. var. *lorifolia* Bailey, l.c. 240

- I. Common turnip. Fig. 49. Our markets offer two classes of common turnip, the one a yellow-, cream- or white-fleshed type of medium or large size and commonly used both for the table and for cattle feed, and the other a white, soft-fleshed, small type used almost exclusively for table use, sometimes referred to as the garden turnip and appearing early in the fall. The former closely resembles the Swede turnip but may be distinguished from it by the fact that its bulb is an enlarged root and hypocotyl whereas the Swede turnip contains also a portion of the enlarged stem. This difference may be illustrated by cutting the full grown schizocorm in halves vertically and noting the point where the root joins the stem.

Seed— $1.72 \times 1.56 \times 1.56$  mm. reaching up to 1.84 mm.; *seed coat distinctive*, Figs. 7, 8; Sanford's brown and auburn, fuscous, fuscous black and deep mouse gray; viewed from above oval to round slightly flattened on radicle side; from the side round or oval with the hilum often projecting well above the seed; raphe overlapping the raised, round, medium small, black hilum; slight depression between micropyle and hilum; micropyle long, narrow, and brown.

Cotyledons— $9.4 \times 4.76$  mm.; deep, full, yellow-green later becoming chromium green; dull; thin; smaller in the fall turnip.

Seedling—slender and strong; Tables I and II.

Seedling leaves—thin, develop early; Scheele's green; shiny; soft; more or less wrinkled with sunken anastomosing veins that lose themselves in lateral veins only toward the tip while in annual rape the larger portion of the central vein stops abruptly near to the petiole; setose with soft hairs on lamina often bent to point toward the tip as if blown by a wind; oval; irregularly triserrate.

II. Japanese foliage turnip, shogoin, tennoje. Figs. 50 to 54 *a*. Leaves sold as greens in spring, bulb forming in the fall.

Seed— $2.16 \times 1.84 \times 1.6$  mm.; quaker drab, deep and dark quaker drab; testaceous, cocoa and walnut brown; vinaceous drab, deep and dark vinaceous drab, and grayish-brown; *celled outer subepidermal row* in radial section of seed coat; distinguished from *B. pekinensis*, which it resembles, by the large round raphe, by the general shape, and by the view from above, which shows a clear-cut isosceles triangle; primary net shallow in regular rows.

Cotyledon— $1.59 \times 1.1$  cm.; apple green; bright; medium thick; angular.

Seedling—sturdy; tall; primary leaf rapidly elongating; slightly thickened.

Seedling leaves—glabrous or sparsely setose on edges; forest green becoming lighter and brighter; deep veins, the central noticeably broadened; spatulate with lamina forming a ribbon down petiole; coarsely, evenly, and shallowly serrate.

III. Strap leaf turnip. Fig. 51.

Seed— $1.68 \times 1.6 \times 1.48$  mm.; deep and dark purplish-gray, Hay's russet and liver brown, and a few seeds of dark purplish-drab, burnt umber and chocolate brown; covered with gray mucilage in contrast to pak toy, which it resembles; viewed from above rectangular, raphe almost outside the small, round black hilum, from the side elongated from top to bottom and flattened and curved on the lower left corner; seed coat resembling that of common turnip.

Cotyledon— $6.35 \times 7.9$  mm.; light bice green; dull, medium thick; central vein prominent; indentation shallow and angular.

Seedling—fine; small; slender; red tinge to stem and petiole.

Seedling leaves—light bice green later becoming Rinneman's green; shiny; wrinkled; veins white, deeply sunken; not glaucous; bristly setose with very short hairs; broadly oval with truncate tip; evenly biserrate, resembling that of Swede turnip.

B. *Brassica Rapa* L. var. *septica* Bailey, l.c. 240

Seven-top turnip. Fig. 52. Tender sprouts are sold in the spring but later in season a bulb forms.

Seed— $1.6 \times 1.4 \times 1.2$  mm.; neutral gray, deep neutral gray, deep brownish-and dusky drab, dark gray, sorghum and Hay's brown; viewed from above wedge-shaped, distinct radicle ridge, from the side slightly flattened at top of upper right corner and considerably flattened at lower left; hilum very small, oval, white tissues and mucilage extending across it tend, in appearance, to cut seed in half; primary net shallow.

Cotyledon— $4.76 \times 7.9$  mm. but on opening only about  $2 \times 1$  mm. in size; turtle green; dull; medium thick; central vein prominent; heart-shaped; indentation shallow and angular.

Seedling—fine; small; stem white.

Seedling leaves—Schafe's green; rugose; thick; leathery; setulose with bristly hairs; veins deeply cut; slightly more elongated ovate than common turnip; regularly shallowly serrate.

C. *Brassica Rapa* L. var. *periviridis* Bailey, l.c. 243

*B. campestris* L. var. *chinensis* (L.) Kondo, *Brass. Spec. in Japan* (1917)

Tendergreen or mustard spinach, komatsu-na. Fig. 54 c. This plant has been placed in this group on account of its slightly thickened pure white tap root but in Eastern Asia it is usually included, with the other "sprouts" or "na's" in *B. campestris* L. Perhaps that is the better placing. The plant itself is used for greens, while in China in the fall the roots are cut in 1.8 cm. cubes, tied in bundles of six or eight and pickled. In this form they were imported into this country and offered for sale on our markets.

Seed— $1.8 \times 1.72 \times 1.48$  mm.; dark vinaceous and dark grayish-brown with a few quaker drab seeds; viewed from above an isosceles triangle slightly rounded at the apex and with the micropyle at the center of the base more as in the common turnip than as in Chinese cabbage, from the side triangular with the right side perpendicular, the lower left corner cut away and the top and other side but slightly rounded; primary net varying; secondary net just visible.

Cotyledon— $1.27$  cm.  $\times$   $9.5$  mm.; forest green; bright; central vein distinct; indentation deep and curved.

Seedling—low; sturdy; *lumiere green stems*.

Seedling leaves—forest green or lighter in color; glazed; sunken lateral veins; may or may not have a cluster of setose hairs at the slightly enlarged tip of serrations and scattered hairs on edges; broadly oval to round, very slightly acuminate; exceptionally shallowly serrate, almost entire.

13. ***Brassica chinensis*** (L.) Bailey, l.c. 253

*B. campestris* L. subsp. *chinensis* L. var. *brassicata* (L.) Burkill, l.c. 1

*B. Napus* L. var. *chinensis* (L.) Schulz, l.c. 45

Pak choi, white celery mustard. Fig. 54 *b*. This plant in the green state is commonly sold in Canada by Chinese merchants for use as vegetable. It somewhat resembles Swiss chard in its broadened white veins and petioles, which, forming a loose head at the base, lose themselves by dividing and projecting fanwise far into the lamina. This plant has been placed in *Brassica chinensis* L. but possibly it might better have been included in *B. campestris* L.

Seed— $1.6 \times 1.28 \times 1.48$  mm.; deep and dark neutral gray, dark vinaceous, seal brown, and dark grayish-brown; viewed from above rounded, sometimes oval, with a *small raphe* that differs from the large one of *B. pekinensis* Rupr.; *hilum small round and black* like that of common turnip, from the side rounded except for the slight flattening on the lower right side and the decided one on the upper right corner; primary net fine, shallow, and in rows; only slight trace of vinaceous mucilage; micropyle long and narrow.

Cotyledons— $7.9 \times 4.76$  mm.; rivage green; dull; thin; central vein prominent; indentation broad, shallow, rounded in one, narrower, more triangular in the other.

Seedling—small; white succulent stem tinted red.

Seedling leaves—smooth, glabrous, thick, evenly serrate with a tiny sawlike hair at the point of each serration; obovate acuminate; lamina ribbon down stem for short distance.

14. ***Brassica campestris*** L. Spec. pl. ed. I, II (1753)

*B. Rapa* L. var. *campestris* (L.) Koch, Syn. Fl. Germ. et Helv. 1, ed. 55 (1835)

A. *Brassica campestris* L. Sp. pl. 666, Fig. 128, 130 (1753); Bailey l.c. 245

*B. Rapa* L. var. *sylvestris* Lam. f. *campestris* (L.) Koch; Hegi l.c. 259

Wild *campestris*, field mustard. Figs. 53 to 56 *a*, Figs. 11, 12. Seed gathered from plants growing wild in eastern Canada was identical with that of a sample of bird feed seed cultivated in Argentina and imported into Canada except that raised micropyle was not pronounced.

Seed— $1.48 \times 1.4 \times 1.36$  mm.; dull; deep, dark and dusky purplish-gray with some seal brown and dark purple drab; viewed from above almost a square with rounded corners and radicle ridge very slightly visible; from the side, almost round; raphe, with vascular bundle scar appearing as a small white dot, is on well defined oval ring of the brownish-black hilum; micropyle on tip of *extended radicle ridge* causes a depression between it and the raphe not found in the smaller seeded strains of turnip; primary net, distinct with fine mesh, is arranged in circles around raised micropyle.

Cotyledon— $8.5 \times 4.76$  mm.; medium green; indentation narrow.

Seedling—small; first two leaves appearing simultaneously.

Seedling leaves—small; dull; thick; sparsely setulose on upper lamina, under veins, and petioles; elliptical to round often narrowing toward the tip; small, serrated end lobe; primary often with three serrations on one side and four on other; secondary with four biserrations; later leaves becoming cut.

- B. *Brassica campestris* L. f. *biennis* Reichb. Deutschl. Fl. I. t. 92 Fig. 4434 B. 106 (1837-38); Syn. Hegi. 261

*B. campestris* L. *autumnalis* DC. Syn. Schulz l.c. 49

*B. Rapa* L. var. *sylvestris* Lam. f. *biennis* (Metz.) Alef. Hegi. l.c. 261

*B. Rapa* L. *oleifera biennis* Metz. Syst. Besch. cult. Kohlart 50 (1833)

- I. Roller rape, oil rape. Fig. 55. Imported as bird feed. Prize roller canary and other bird fanciers demand this seed when possible claiming they can distinguish it by its sweet, mellow, nutty flavor, and by the way birds enjoy it.

Seed— $1.7 \times 1.7 \times 1.55$  mm.; aniline black, Sanford's brown, deep and dark livid brown, Morocco red, dark and dusky purplish-gray; viewed from above circular with conspicuous round black hilum and small raphe partly within it; from the side, all corners but lower left are angular and seed is often elongated; seed coat *almost smooth, bright*; primary net shallow, small, round-meshed; hilum, biennial with its cells distinct from those of seed coat and with two strands of mucilage from raphe circling its central scar. Figs. 13, 14.

Cotyledons— $7.9 \times 6.35$  mm.; Rinneman's green; dull; medium thin with prominent midvein; angled near the base of lamina; indentation narrow and arcuate.

Seedling—strong; no internode; leaves whorled; Table I.



Seedling leaves—large; dull; not glaucous; rugose; hirsute above, below, on edges and on petiole; primary elliptical; unsymmetrical; lamina at base acuminate on one side, on other broadly cut at an angle with petiole to form chin of a caricature of a human profile; broadest in center; four or six uneven coarse serrations; later leaves becoming lyrate, with three small segments and three lobes in main section.

C. *Brassica campestris* L. f. *annua* Reichb. l.c. Fig. 4434 a; Syn. Hegi. 260

*B. Rapa* L. var. *sylvestris* Lam. f. *annua* (Metz.) Alef.; Hegi. l.c. 260

*B. Rapa* L. *oleifera annua* Metz. Syst. Des. Kohl. 51 (1833)

I. Bird rape, red summer rape, Fig. 27. Low grade bird feed. Seeds of wild Brassicas as well as cultivated, of low vitality, are commonly present. Extreme forms grow quickly, branch readily, mature early, and produce an enormous number of seeds. Indeed some produce seed in the garden at a height of four inches. This may be the plant described by Muravieva (1928 (41)) as *B. campestris*, field cabbage.

Seed— $2.1 \times 1.9 \times 1.7$  mm.; dull; Sanford's brown, dark livid brown and dusky drab, Hay's brown, russet and liver brown; viewed from above cubical with rounded corners, flattened, slightly less on radicle side; from the side, oval, lower left corner cut away so that seed rests naturally there; primary net, large-meshed, exceedingly shallow, with straight, flat, and broad walls.

Cotyledon— $1.43 \times 1.27$  cm.; medium green, dull, thick, indentation arcuate twice as wide as deep.

Seedling—elongating rapidly; may flower after third leaf.

Seedling leaves—very shiny; forest green; usually glabrous sometimes sparsely setose on under veins or upper lamina; oblong; acute angled at base; regularly serrate.

In the case of all annual rapes if primary or secondary leaf shows an unusual *five-lobed* or serrated *juncea* character, pure seeds of *B. juncea* (L.) Coss. can be expected to segregate out. Closely related strains produce larger plants. Their seeds are smaller, with a deeper-walled small-meshed net and seedling leaves are duller, broader, and softer in texture.

II. Rubsen rape, small red rape. Fig. 58. A strain much used in bird feed. Easily identified by seed.

Seed— $1.9 \times 1.8 \times 1.4$  mm., deep livid brown, Hay's brown, warm blackish-brown, deep brownish-drab. Viewed from above ovate to wedge-shaped, always flattened at hilum, from the side, flattened to straight line on right, with top curved slightly upward and left side and base forming a sweeping curve; net fine and irregular, seed coat reticulated. Figs. 9, 10.

Cotyledon—1 cm.  $\times$  7 mm.; other slightly smaller; dull, thick; chromium green; midvein distinct; indentation angular.

Seedling—internode; forest green stem tinted red.

Seedling leaves—soft; slightly wrinkled; Rinneman's green; glabrous; broadly elliptical with four irregular serrations.

III. Sweet bird rape. Fig. 59. Imported as bird feed, and rarely for oil. Wide variation.

Seed— $1.8 \times 1.6 \times 1.5$  mm.; Hay's russet, Morocco red, liver, dark vinaceous, seal, dusky, and blackish browns. Viewed from above, elliptical with an annular hilum, slightly darker than seed coat, raised in line with curved top of seed; micropyle brown; from the side, elliptical to shield-shaped with upper right top flattened; coat with reticulations elongated lengthwise, secondary net, clear, of fine round mesh. Netting often deeper, larger, and more pronounced.

Seedling—tall, strong, short internode.

Seedling leaves—smooth, setulose above, below, and on petioles; elliptical; truncate tip; each side cleft into four varying, irregular, unsymmetrical lobes or serrations; sometimes erose.

15. ***Brassica elongata*** Ehrh. Beitrage Naturh. VII. 159 (1792)

Seed— $1 \times 0.75 \times 0.66$  mm.; olive gray with very distinctive strip of Natal brown covering the radicle ridge, the tip of which projects above the seed. When seed is crushed and heated with chloral hydrate, the outer kernel skin colors red but the seed coat, although it closely resembles in texture that of *B. arvensis* (L.) Rabenh., does not bleed.

16. ***Brassica juncea*** (L.) Coss., Bull. Soc. Bot. Fr. VI. 609 (1859)

Salad plants grown in gardens and some oil seeds produced.

Seeds of the species were well defined and had a definite heavy netting. *B. juncea* represents an exceedingly large group. Seeds from different sources were grown and the resultant plants showed astonishing differences, varying in height from a few inches to several feet and otherwise varying from small tender plants to very large and coarse rushlike ones, which might or might not be leafy and branching. The salad forms are the curled and broad leaved varieties plain or savoy type. Seed distinction within the species is difficult.

A. *B. juncea* (L.) Coss., wild form

Brown mustard, wild mustard. Fig. 63. In several samples of grain screenings from Western Canada this wild *juncea* seed constituted somewhat less than 1% of the *small* seeds. The percentage is much smaller in Ontario screenings.

**Seed**— $1.6 \times 1.52 \times 1.4$  mm.; dark Natal brown and bone brown; often perfectly round but sometimes elongated, net shallow and superficial. Distinguished by the very tall-celled outer epidermal row that appears when radial section of seed coat is immersed in water. Fig. 15.

**Cotyledons**— $1.56 \times 1.27$  cm.; Rinneman's green; slightly American green glaucous; dull; medium thick; edges and prominent central vein tinted red; cordate with distinct angle at base of side; indentation deep, narrow, and curved.

**Seedling**—sturdy; green stem with bronze tint; *no marked tendency to whorl*; Table I.

**Seedling leaves**—large; shiny; smooth; *very dark green*; veins sunken; sparsely setose on veins, edges, and petiole; very rarely symmetrical; fragments of lamina usually but not always appearing down the stem; in other cases base of the lamina parted; oblong with slight truncate tip.

## B. Leaf mustards

### I. *B. juncea* var. *crispifolia* Bailey, l.c. 41. (1922)

Curled mustards. Fig. 61. Common strains; curled mustard, giant southern curled, and ostrich plume.

**Seed**— $1.44 \times 1.4 \times 1.36$  mm.; vinaceous and dark vinaceous brown, deep brownish- and dusky drab and a few deep and dark mouse gray; viewed from above circular, from the side egg-shaped; net comparatively *shallow* and of *medium-sized mesh*. Radial section in water shows medium tall-celled outer epidermal row.

**Cotyledons**— $1.27$  cm.  $\times$   $9.5$  mm.; *ambrical green*; glaucous; dull; thick; slight angle at base giving a more rectangular appearance than that of the average heart-shaped *juncea* cotyledon; central vein only; indentation irregular.

**Seedling**—medium fine; greenish-white stems.

**Seedling leaves**—characteristically *shiny*; sparsely setose with short hairs on edges of primary leaf and on lamina in secondary leaf; some much more elaborately cleft or parted than others.

### II. *B. juncea* var. *foliosa* Bailey, l.c. 263 (1930)

Common strains—Florida broad leaf, elephant's ear, chirimen savoy leaf, new Chinese and Chinese smoothleaf mustards, Fordhook fancy. Fig. 16.

#### a. Florida broadleaf; smooth and Savoy types, some cut and curled.

**Seed**— $1.56 \times 1.48 \times 1.4$  mm.; mixture of vinaceous and dark vinaceous brown, deep brownish- and dusky drab, buff and olive browns; viewed from above rectangular, from the side showing an elongation on the upper left side topped by a raised micropyle; net large with deep perpendicular walls. Section of seed coat mounted in water showed only a trace of shallow cells in outer epidermis.

Cotyledons— $9.5 \times 4.7$  mm.; Rinneman's green; glaucous American green tint; translucent; flattened heart-shaped with lamina ribbon down stem.

Seedling—medium; white stem with red tint or green stem with bronze tint; petiole grooved. Fig. 62.

Seedling leaves—setose; shiny; lighter green; veins white; pronounced; elongated, oblanceolate, ribbon lamina down petiole for a short distance; irregularly, coarsely and shallowly serrate.

b. Guy toy. Fig. 60.

These plants as greens may be purchased from Chinese merchants and are recommended because of their more pungent flavor.

Seed— $1.32 \times 1.28 \times 1.2$  mm.; mixture of brownish- and dusky drab; liver, Hay's, and chocolate brown; net small-meshed.

Seedling—considerable variation.

c. Taka-na, a Japanese mustard.

Seed— $1.5 \times 1.3 \times 1.3$  mm. in size, and haematite red, Prussian red, dark vinaceous drab, and dark grayish-brown in color.

C. Oil producing mustards. Brown mustards.

Samples of seed were received from European universities. The similarity of the seeds of different strains gave little clue to the variations in the mature plants, some of which, growing much branched and rankly to a height of 4 ft., seemed to justify the name "*juncea*", which is derived from the latin "*juncus*" meaning "rushlike". Schulz (l.c. 55) points out two varieties of *juncea* seeds, variety *lutea* Batalin, honey yellow, and variety *fusca* Batalin, darker in color. In the lighter colored oil group the seeds of *fusca* are Hay's russet, liver brown, with a few dusky drab and vinaceous brown. The oil seeds usually have a thick-walled heavy net of large mesh through which is seen the alveolate seed coat. Radial sections of the seed coat show an *uncelled thick subepidermal row*.

It is in this group that Burkill places *B. Besseriana* Andr. with its large oval dark brown seeds and also the "Rai" of India and *S. ramosa* Roxb.

17. ***Brassica integrifolia*** (West) Schulz, ap. urb. Symb. antill. III. 3. 509 (1901)

Fig. 64. Not common.

Seed— $1.2 \times 1.2 \times 1.5$  mm.; honey yellow; brown hilum; raised white raphe; small brown micropyle; primary and secondary nets straight-sided, distinct.

Cotyledons—1.12 cm.  $\times$  8 mm.; dark blue green; hypocotyl red; petiole elongated; cotyledon heart-shaped with flattened sides; indentation shallow, narrow, and arcuate.

Seedling—tall; *strong*; *coarse*; stem green with purple tint.

Seedling leaves—dark with purple tint; whorled; wrinkled; net-veined; setulose above and below; obovate; broadly and unevenly dentate; fragments of lamina down petiole.

18. ***Brassica nigra*** (L.) Koch, in Rühling's *Deutschl. Fl.* ed. 3, IV. 713 (1833)

Black mustard. Fig. 66. Seeds of black *B. arvensis* type are sometimes incorrectly called black mustard. These are not included in this paper. Six varieties of *B. nigra* are listed by Schulz (1919) but are not grown to any extent here, nor are the seeds of *B. nigra* a common impurity in our *Brassica* seeds.

Seed—1.58  $\times$  1.3  $\times$  1.2 mm.; ranging from 1.44 to 1.6 mm.; Fig. 15; color varying according to variety from dark red to the buff brown of the *juncea*; viewed from above circular, flattened at micropyle, from the side irregular, elongated, and often simulating other *cruciferous* seeds more than *Brassica*; net deep-meshed covering the seed and if removed would leave the seed coat smooth and shiny.

Cotyledon—6.35  $\times$  8 mm.; deep dull yellow-green with the typical tint of American green; thick; smooth; heart-shaped; indentation arcuate.

Seedling—fine; small; decided internode; stem glabrous.

Seedling leaves—deep; dull, yellow-green; glabrous or more or less setose; waxy; straight sunken veins; unsymmetrical; crenately and alternately lobed; oblanceolate to oval.

19. ***Brassica arvensis*** (L.) Rabenh. *Fl. lusatica* I. 184 (1839)

*B. sinapis*trum Boiss. voy. Bot. Midi de l'esp. II. 39 (1839–1845)

Wild mustard. Fig. 65. Of the many varieties of *arvensis* only two growing in our fields and cultivated crops are well known. Fernald has reported finding in eastern Canada another variety, *Schbuhriana* Reichb., which is distinguished by a slender silique 1.5 to 2 mm. thick. No sample of seed of this variety was available.

a. Seed—deep dark dusky gray and Prussian red; coat rougher than Type b; spherical, 1.6  $\times$  1.6  $\times$  1.6 mm.

b. Seed—small; smooth; spherical; russet vinaceous, sorghum brown, Hay's brown, light seal brown; mature portion of the seed coat, if boiled in a solution of chloral hydrate, bleeds, i.e. traces of *red liquid* appear to flow very slowly from the reddened cell; Prussian red seeds of first variety are not sufficiently mature to test until a portion of coat darkens.

Cotyledons— $9.5 \times 6.35$  mm.; medium green; smooth; light glaucous coating of blue when young; larger in black-seeded variety; may or may not show red color; indentation deeply curved.

Seedling—slow growing; sturdy; soon becoming ragged and weedy; stems greenish-white with purple tint; unable to separate two varieties by seedling. Table I.

Seedling leaves—smooth; decidedly pubescent with short hairs above, below, on the edges and petioles; thin, elongated linear to lanceolate; upper third of the leaf narrowing in a series of two or three right-angled steps to the truncate tip; often narrows toward the petiole in the same manner; irregularly entire or crenately repand; biserrate; symmetrical.

20. **Brassica alba** Rabenh. Fl. Lusatia I. 185 (1839)

**Sinapis alba** L.

White mustard; imported for the manufacture of table mustard. Fig. 67.

Seed— $2.25 \times 2.25 \times 2$  cm. with range from 1.5 to 2 cm.; large; honey yellow; porous coat; raphe pronounced from side view; distinguished by cross section of seed coat or by immersing half of the seed in water and marking the behavior of mucilage.

Cotyledons— $1.59$  cm.  $\times$   $7.9$  mm.; leaf green; dull; thick; central vein and two pairs of lateral veins very deeply sunken; indentation broad, semicircular but irregular; petiole pubescent.

Seedling—large; medium fine; pubescent and touched with bronze or purple. Table I.

Seedling leaves—pubescent on petioles, under veins and edges; pinnately lobed.

21. **Brassica dissecta** Boiss. l.c. 40; Schulz syn. 132

Seed—brown; from side view raphe less prominent, otherwise appearing as *B. alba*.

Cotyledons— $1.8 \times 1.1$  cm.; pronounced veins; indentations broad, semicircular; setose on petiole.

Seedlings—similar to *B. alba*.

Seedling leaves—similar to those of *B. alba*, but very coarsely lobed.

22. **Brassica barrelieri** (L.) Janka. in Termesz. Fig. VI, 179 (1882)

*B. subularia* Pers. Syn. II, 207 (1807); Schulz Syn. 65

Seed— $0.75$  mm. in diameter, perfectly round except for the slightly small round hilum capped with mucilage; dark red brown color with a net of hexagonal mesh so large that only 9 or 10 meshes reach across the seed.

# KEY FOR DISTINCTION OF *Brassica* SEEDS BY MEANS OF THE SEED AND OF SEED COAT STRUCTURE

## For placement of seed:

1. Viewed from above with micropyle, raphe, and hilum visible.
2. Viewed from side with radicle at left and raphe above the micropyle and the hilum.

NOTE: *Brassica* seeds are identified only by the intimate knowledge and the segregation of a multiplicity of detail.

## a. Seeds honey yellow in color.

- b. Seed coat heavily netted, under 1.3 mm. dia. .... 17. *B. integrifolia* (West) Schulz.
- c. Seed over 1.3 mm. dia. .... 16. *B. juncea* (L.) Coss.
- b. Seed coat porous; glaucous; seed dia. over 2 mm. .... 20. *B. alba* (L.) Rabenh.
- b. Seed coat porous; not glaucous; seed under 2 mm. dia.;  
oleraceous radicle and shape ..... 3B. *B. oleracea* L. var. *acephala* DC.
- b. Seed coat reticulated ..... 14. *B. campestris* L.

## a. Seeds darker than honey yellow.

- d. Seed coat porous; glaucous; dull cinnamon brown; over  
2 mm. dia. .... 21. *B. dissecta* Boiss.
- d. Seed coat smooth; palisade cells tall, narrow, tightly  
packed, and of uniform height.
- e. Palisade cells of seed coat *not* bleeding in chloral hyd-  
rate; seed spherical, approx. 1 mm. dia.; dull; strip  
of brown color covering the radicle ..... 15. *B. elongata* Ehrh.
- e. Palisade cells of seed coat bleeding in chloral hydrate;  
seed spherical or elongated; dull dark dusky gray or  
shiny purplish-gray (two varieties) ..... 19. *B. arvensis* (L.) Rabenh.
- d. Seed coat prominently netted.
- f. Seed usually elongated; primary net in seed coat with  
slanting walls, seed coat below net, shiny, smooth;  
from side, seed falls away beneath the micropyle to  
form a perpendicular radicle ridge ..... 18. *B. nigra* (L.) Koch.
- f. Seed tending to spherical; raphe raised; primary net  
on seed coat with narrow straight-sided walls usually  
mucilage-tipped; seed coat below dull, alveolate;  
from side, seed beneath micropyle full and rounded ... 16. *B. juncea* (L.) Coss.
- g. Subepidermal layer of radial section crushed but in  
water an outer row of shallow cells appears ..... 16B. *B. juncea* (L.) Coss.
- g. Subepidermal layer in water shows an outer row of  
deep cells ..... 16A. *B. juncea* (L.) Coss.
- d. Seed coat neither smooth, porous, or prominently netted.
- h. Seeds vinaceous, glaucous, from above, smooth, wedge-  
shaped with rounded sides; from side, seed slants  
from the *large* raphe forming an elbow near the  
middle or in the upper half of the right side.
- i. Seeds over 1.5 mm. in breadth; reticulations very  
fine, shallow, round, irregular; crushed subepi-  
dermal layer; special bodies in the pigment layer ... 9. *B. pekinensis* Rupr.
- i. Seeds over 1.5 mm. in breadth; large raphe angling  
over the hilum from a depression; reticulations  
fine, irregular; outer row of subepidermal layer is  
celled ..... 12A. II. *B. Rapa* L. var. *lorifolia* Bailey

- i. Seeds over 1.5 mm. in breadth; from above, large raphe touching the micropyle surrounded by white mucilage; from the side, elbow forming upper right corner; reticulations deep, in rows.....12C. *B. Rapa* L. var. *periviridis* Bailey
- i. Seeds 1.5 mm. in breadth or under; reticulations fine, tending to regular rows, micropyle long, narrow, black.....13. *B. chinensis* (L.) Bailey
- h. Seeds deep dark and blackish mouse gray; 1.9 mm. in breadth; from above, circular; from the side, shield-shaped rounded at top; shallow square-meshed net; spots of gray mucilage.....4. *B. alboglabra* Bailey
- h. Seed from above, brown or gray oleraceous colors either in coat or in mucilage on coat; rough; large, oval hilum, raphe outside.
- j. Radial section of seed coat shows tumbler cells loosely packed, of uniform height, with uniformly thickened walls; outer subepidermal row celled (sheep kale, asparagus kale, and ragged Jack kale excepted) with conical projections of mucilage in center of base of each cell.....3. *B. oleracea* L.

NOTE: Varieties of *B. oleracea* distinguished by shape of seeds (Figs. 18, 19) and by color, as indicated in the following portion of the key.

- k. Deep mouse gray, spherical.
- l. Large.....3B. *acephala* DC.  
Ragged Jack kale  
Asparagus kale
- l. Small.....3B. *acephala* DC.  
Sheep kale
- k. Seeds of clay color and tawny olive conspicuously present.....3B. *acephala* DC.  
Green kale  
Variegated kale
- k. Seeds of Saccardo's umber and sepia, mouse gray, brownish-drab with the occasionally warm sepia, russet, or hazel; seed coat very thin.....3E. *gemmifera* (DC.) Zenk.
- k. Seeds of sorghum brown; Hay's brown and light seal brown, shades of brownish-drab, Morocco red, claret brown, and mouse gray.....3H. *gongylodes* L.
- k. Seeds of drab and hair brown, of Natal and bone brown.....3G. *botrytis* L.  
*cauliflora* (Gars.) DC.
- k. Seeds of hazel and chestnut brown, of Natal brown and bone brown, few deep olive gray, and wood brown.....3G. *botrytis* L.  
*asparagoides* DC.
- k. Seeds of dark olive buff, mouse gray, dark olive gray, olive brown, and clove brown.....3B. *acephala* DC.  
Marrow kale
- k. Seeds of grayish olive, olive gray, olive brown, and clove brown.....3A. *ramosa* (DC.) Alef.
- k. Seeds of vinaceous drab, fuscous and fuscous black, pecan brown, and Rood's brown.....3D. *sabauda* L.
- k. Seeds of testaceous, cocoa brown, walnut brown and burnt umber, deep brownish drab and dusky drab, and blackish brown, fuscous, and a few dark olive gray.....3F. *capitata* L.



- h.* Seeds uniformly purplish, neutral or mouse gray, dusky or blackish-brown; from above, smooth, rectangularly spherical, flattened in hilum region; raphe outside oval hilum.
- j.* Radial section of seed coat with cells of subepidermal layer crushed; tumblers with flared brims heavily lignified above and turned back and downwards to form a depression at junction of two brims; lumen of tumbler broader than double wall.
- m.* Cotyledons grown in dark, large, empire yellow in color.....10. *B. Napus* L.
- n.* Seeds spherical; no anthocyanins in seedlings; radial section with irregularly broad double tumbler walls with marked depression; see seedling.....10A. *B. Napus* L. f. *biennis* Thell.
- n.* Seeds spherical; no anthocyanins in seedlings; radial section with narrower, taller, double tumbler walls and narrower, deeper depressions; see seedling.....10B. *B. Napus* L. f. *annua* Thell.
- n.* Seeds cubical, resting naturally with hilum above; purple and bronze anthocyanins in seedling; see seedling.....11. *B. napobrassica* (L.) Mill.  
White-fleshed Swede  
turnip
- m.* Cotyledons grown in dark, Naples yellow or chrome in color.....11. *B. napobrassica* (L.) Mill.  
Yellow-fleshed Swede  
turnip
- h.* Seeds from side, a small white raphe angles over the raised round black hilum from the right of which the top slants down to the upper right corner, lower left side flattened; micropyle long, narrow and brown; seed coat characteristic; typical color, fuscous.
- j.* Radial section of seed with the lignified portion of the inside tumbler wall tapering in a graceful curve to a peak at junction of two tumbler brims; outer subepidermal row crushed but not dipping down into the lumen of the tumbler.....12. *B. Rapa* L.
- o.* Seeds with smallest dia. 1.2 mm.; color typical; from above, wedge-shaped; distinct radicle ridge; straight line of micropyle and tissue over raphe and hilum appears to divide seed.....12B. *B. Rapa* L. var. *sephiceps* Bailey
- o.* Seeds over 1.2 mm. dia.; from above, rectangular; confused with *B. chinensis* L.....12A. *B. Rapa* L. var. *rapifera* Metz.  
Strap leaf turnip
- o.* Seeds over 1.2 mm. dia.; from above and from side, oval to round.....12A. *B. Rapa* L. var. *rapifera* Metz.  
Common turnip
- h.* Seed colors Hay's brown, russet, or Morocco red, indicated; seed coat reticulations becoming flat-topped and ranging close to netting; from above, shield-shaped, from side, same, sometimes with slight flattening on right top or lower left.
- j.* Radial section of seed coats, with palisade cells more cylindrical; with the inner wall of the tumbler cells angled where its lignified portion tapers slightly to the brim; outer subepidermal row crushed.....14. *B. campestris* L.

- p.* Seeds approximately 1.7 mm. dia.; bright; regularity of tumbler walls and size of lumen easily noted in simple surface view slide; seed coat with small, medium deep, and irregular primary net, satin finish; indication of Morocco red.....14. *B. campestris* L. f. *biennis* Reichb.
- p.* Seeds almost spherical, 1.4 mm. dia.; sometimes elongated; thin gray glaucous coat; radicle ridge extending above the seed; note surface view slide.....14. *B. campestris* L. f. *annua* Reichb.  
Wild form
- p.* Seeds larger, shield-shaped; typical but of wide variation; tendency to russet and Hay's brown; shallow or deep flat-topped reticulations with fine or coarse mesh; see surface view slide.....14. *B. campestris* L. f. *annua* Reichb.  
Bird rape

KEY TO *Brassica* SEEDLINGS

- a.* Stem decidedly setose, cotyledons large with two pairs of distinctly prominent veins.
- b.* Seedling leaves lyrate, lateral lobes unequally crenate.....20. *B. alba* (L.) Rabenh.
- b.* Seedling leaves parted, lateral lobes broadly dentate.....21. *B. dissecta* Boiss.
- a.* Stem glabrous.
- c.* Cotyledons light green.
- d.* Stems white.
- e.* Seedlings glaucous, oleraceous, seedling leaves olivine green.....4. *B. alboglabra* Bailey
- e.* Seedlings not glaucous or oleraceous; stems and veins white and succulent.
- f.* Cotyledons exceedingly large, acuminate at base, apple green.....9. *B. pekinensis* Rupr.
- f.* Cotyledons small, rivage green.....13. *B. chinensis* L.
- d.* Stems lumiere green.
- g.* Seedling leaves rapidly becoming oblanceolate, lamina ribbon down stem.....12A. *B. Rapa* L. var. *rapifera* Metz.  
Shogoin turnip
- g.* Seedling leaves small, round to oval attenuate at base with short petioles.....12C. *B. Rapa* L. var. *periviridis* Bailey
- d.* Stems apple green—seedling leaves apple green, soon becoming cut.....3B. *B. oleracea* L. var. *acephala* DC.  
Green kale  
3C. Palm tree kale
- c.* Cotyledons medium green.
- h.* Cotyledons fleshy, waxed; seedling leaves with greenish glaucous coat.
- i.* Seedling leaves with the green overshadowed by red, blue, purple, or white.....3B. *B. oleracea* L. var. *acephala* DC.  
Variegated kale  
White ribbon kale

## i. Seedling leaves with no anthocyanins visible.

## j. Cotyledons when young puffing in center like a balloon top.

k. Stems, petioles, and veins succulent, thick; seedling leaf stiffly erect and almost entire..... 3F. *B. oleracea* L. var. *capitata* L.

k. Stems, petioles, and veins not so succulent; finer; seedling leaf with petiole more gracefully arched, serrately cut..... 3D. *B. oleracea* L. var. *sabauda* (L.) Martens

## j. Cotyledons when young with indentations unsymmetrical, one being broad and almost straightly cut, other arcuate.

l. Cotyledons on opening standing perfectly erect with ventral surfaces together; primary leaf round, entire with short petioles..... 3E. *B. oleracea* L. var. *gemmifera* (DC.) Zenk.

l. Cotyledons opening not standing perfectly erect; primary leaf with petiole gracefully elongated... 3H. *B. oleracea* L. var. *gongylodes* L.

j. Cotyledons when young perfectly circular except for the indentation and junction with the petiole... 3B. *B. oleracea* L. var. *acephala* DC.  
Marrow kale

## j. Cotyledons when young forming a rectangular table.

m. Table with angular pieces cut from each of four sides..... 3G. *B. oleracea* L. var. *botrytis* L. subvar. *cauliflora* (Gars.) DC.

m. Table with more rounded pieces cut from each of four sides..... 3G. *B. oleracea* L. var. *botrytis* L. *asparagoides* DC.  
Broccoli (Walcheren)

## j. Cotyledons with none of these characters.

n. Seedling leaves setose..... 3B. *B. oleracea* L. var. *acephala* DC.  
Asparagus kale

## n. Seedling leaves smooth.

o. Seedling leaf irregular; elliptical; petioles and base of central vein enlarged..... 3B. *B. oleracea* L. var. *acephala* DC.  
Collards

o. Seedling leaf irregular; ovate; petioles and base of central vein not enlarged..... 3G. *B. oleracea* L. var. *botrytis* L. *asparagoides* DC.  
Sprouting broccoli

o. Seedling leaf irregular; broadly cuneate; veins not enlarged..... 3A. *B. oleracea* L. var. *ramosa* Alef.  
Thousand-headed kale

## k. Cotyledons neither fleshy nor waxed but leaflike.

## p. Cotyledons thin; seedling leaves American green, glaucous.

q. Seedling leaves crenately and irregularly lobed; lyrate..... 18. *B. nigra* (L.) Koch.

q. Seedling leaves commonly with five serrations or lobes; often whorled; cotyledons obcordate..... 16. *B. juncea* (L.) Coss.

- g. Seedling leaves symmetrical, diminishing from center toward each end in a series of irregular, broadly obtuse angles; becoming weedy-----19. *B. arvensis* (L.) Rabenh.
- p. Cotyledons thin; seedling leaves not glaucous.
- r. Cotyledons on opening second smallest of the Brassicas (2 mm. diam.)-----12B. *B. Rapa* L. var. *septicaps* Bailey
- r. Cotyledons on opening larger; seedling leaves with four serrations or lobes on each side.
- s. Seedling leaves with some hairs bent in center to form a right angle-----12. *B. Rapa* L.
- s. Bent hairs may or may not be present, seedling leaves appearing early wrinkled, one or more dentations or crenations are combined with serrations in characteristic pattern-----12. *B. Rapa* L. var. *rapifera* Metz.  
Common turnip
- s. No bent hairs present; seedling leaves smooth.
- t. Seedling leaves shiny, flowering early, decided internode-----14C. *B. campestris* L. f. *annua* Reichb.  
Large bird rape
- t. Two seedling leaves appearing simultaneously; dull; secondary leaf biserrate, later leaves incised-----14A. *B. campestris*  
Field mustard
- t. Seedling leaves unsymmetrical, four irregular lobes on each side-----14C. *B. campestris* L. f. *annua* Reichb.  
Summer rape
- r. Cotyledons on opening larger, seedling leaves with four and six serrations, later leaves elongating and becoming lyrate; decidedly setose-----14B. *B. campestris* L. f. *biennis* Reichb.  
Roller rape, oil rape
- p. Cotyledons thick; tough; translucent; distinct pair of lateral veins; seedling leaves gray glaucous.
- u. Seedling leaves only slightly gray glaucous; wrinkled, commonly with six groups of serrations; no internode.
- v. Primary seedling leaf broadly oval to round; biserrate; hirsute-----11. *B. napobrassica* (L.) Mill.
- v. Primary seedling leaf gray glaucous below, bright green above; elliptical with truncate tip; serrations singly or in groups; setose; lamina ribbon down stem-----12A. *B. Rapa* L. var. *rapifera* (Metz.) Bailey  
Strap leaf turnip
- u. Seedling leaves with heavy, gray, glaucous coat; smooth; three or four serrations or lobes; internode; primary seedling leaf with miter-shaped base capped with a prominent dome.
- w. Cotyledons large, resembling two circles touching at midrib; primary leaf generally with no segments down stem; elongated dome; broadest in center-----10. *B. Napus* L. f. *annua* Thell.  
Winter oil rape
- w. Cotyledons large, almost as long as wide; primary seedling leaf generally four-lobed; segments down stem; flattened dome; broadest at top of base-----10. *B. Napus* L. f. *biennis* Thell.

## c. Cotyledons very dark green.

- α. Cotyledons kidney-shaped; seedling leaves sharply, unevenly, serrately cut; glabrous; ovate..... 6. *B. insularis* Moris
- α. Cotyledons heart-shaped.
  - γ. Cotyledons large; elongated; seedling leaves with repand edges..... 7. *B. cretica* Lam.
  - γ. Cotyledons small..... 2. *B. macrocarpa* Guss.
- α. Cotyledon neither kidney- nor heart-shaped.
  - α. Indentation wide almost straightly cut..... 1. *B. balearica* Pers.
  - α. Indentation not so wide, more arcuate.
    - Z. Seedling leaves glaucous blue above, hoary gray beneath..... 8. *B. incana* Ten.
    - Z. Seedling leaves rectangular with gray, glaucous petioles..... 5. *B. Robertiana* Gay.

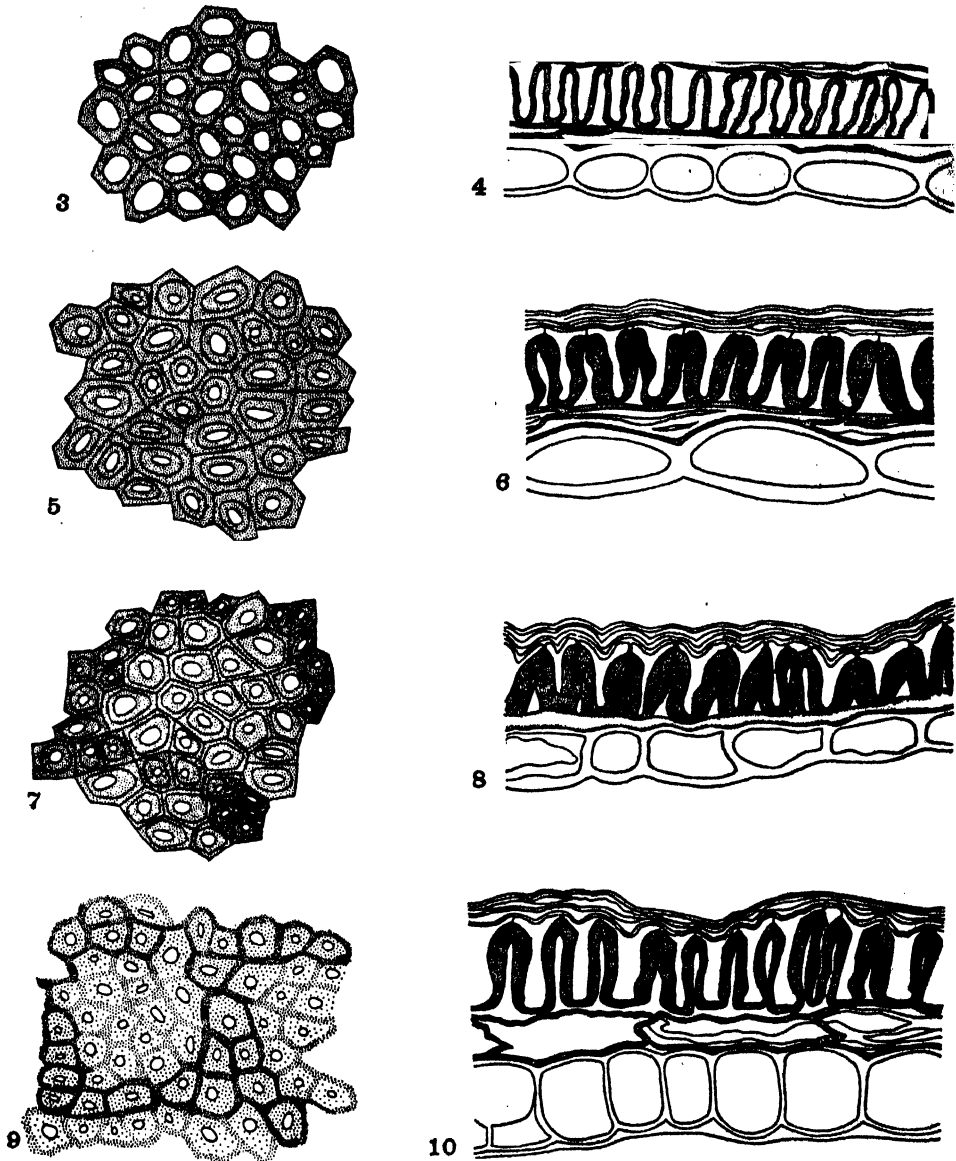
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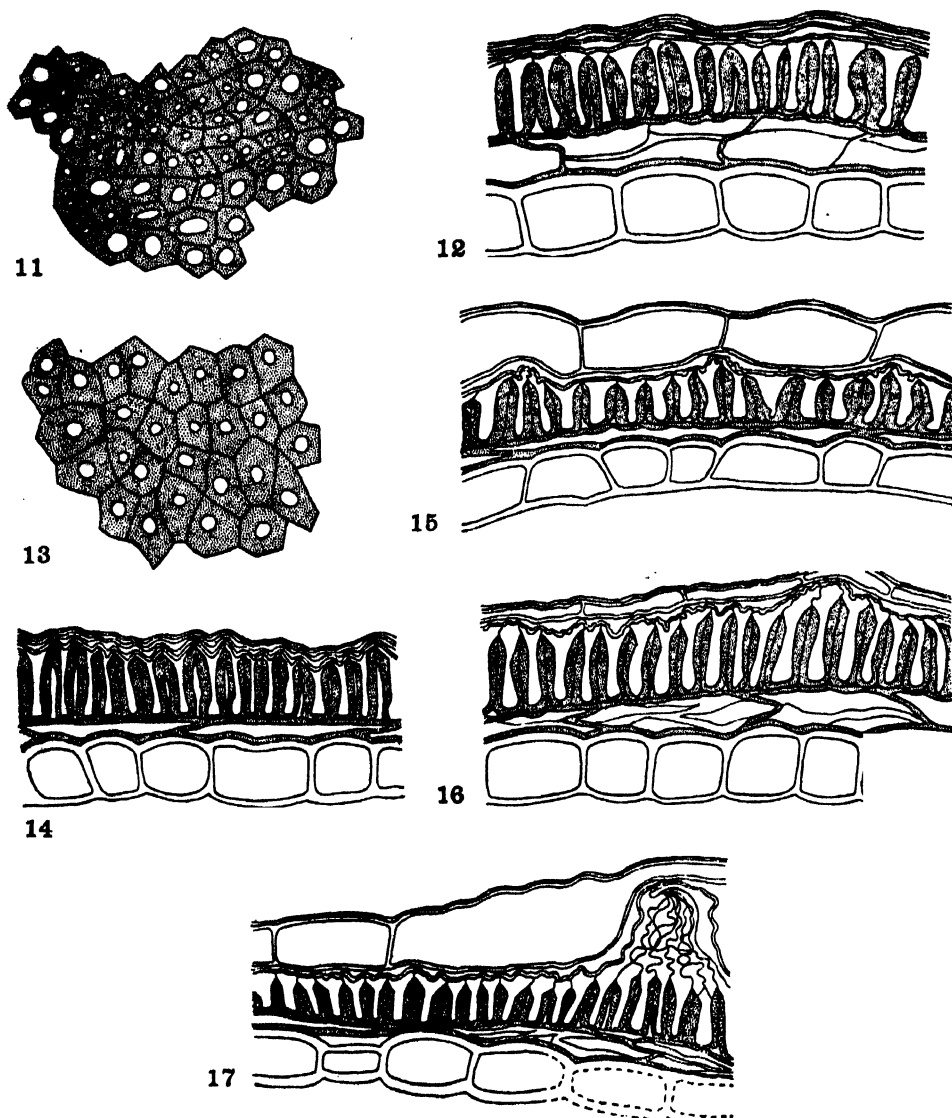
NOTE: Figs. 3-67 will be found on pp. 579-587.



FIGS. 3 to 10 are camera lucida drawings of *Brassica* seed coats ( $\times 280$ ).

- FIG. 3. *B. oleracea* L. var. *gemmifera* (DC.) Zenker. Brussels sprouts. Surface view.  
 FIG. 4. *B. oleracea* L. var. *gemmifera* (DC.) Zenker. Brussels sprouts. Radial section.  
 FIG. 5. *B. Napus* L. var. *arvensis* (Lam.) Thell. f. *biennis* (Shubl. et Mart.) Thell. Dwarf Essex rape. Surface view.  
 FIG. 6. *B. Napus* L. var. *arvensis* (Lam.) Thell. f. *biennis* (Shubl. et Mart.) Thell. Dwarf Essex rape. Radial section.  
 FIG. 7. *B. Rapa* L. var. *rapifera* Metz. Devonshire turnip. Surface view.  
 FIG. 8. *B. Rapa* L. var. *rapifera* Metz. Devonshire turnip. Radial section.  
 FIG. 9. *B. campestris* L.  $\alpha$  *annua* Reichb. Annual rape. Surface view.  
 FIG. 10. *B. campestris* L.  $\alpha$  *annua* Reichb. Annual rape. Radial section.





FIGS. 11 TO 17 are camera lucida drawings of *Brassica* seed coats ( $\times 280$ ).

- FIG. 11. *B. campestris* L., wild form. Radial section.  
 FIG. 12. *B. campestris* L., wild form. Surface view.  
 FIG. 13. *B. campestris* L.  $\alpha$  biennis Reichb., biennial rape. Surface view.  
 FIG. 14. *B. campestris* L.  $\alpha$  biennis Reichb., biennial rape. Radial section.  
 FIG. 15. *B. juncea* (L.) Coss., wild form. Radial section.  
 FIG. 16. *B. juncea* (L.) Coss., smooth leaf form. Radial section.  
 FIG. 17. *B. nigra* (L.) Koch. Radial section.

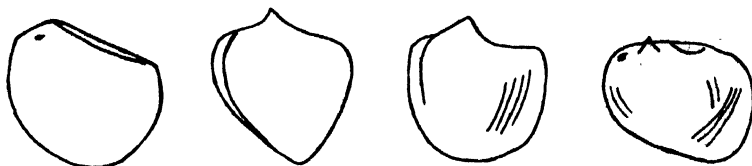
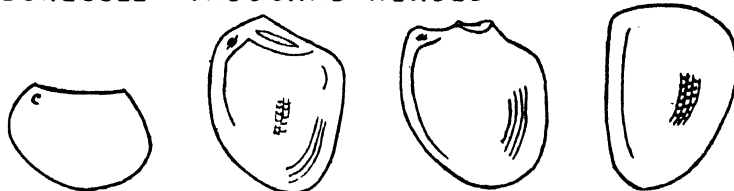
**BRUSSELS SPROUTS****BORECOLE MARROW STEM****BORECOLE THOUSAND HEADED****COLLARDS****BORECOLE WHITE RIBBED WINTER**

FIG. 18. Lateral view of the following varieties and strains of *Brassica oleracea* L., illustrating representative shapes on which distinction of seeds may be based.

Brussels sprouts. *B. oleracea* L. var. *gemmifera* (DC.) Zenker.

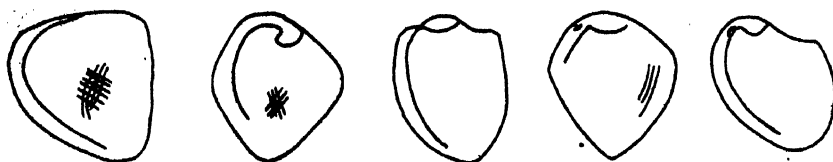
Marrow borecole. *B. oleracea* L. var. *acephala* DC.

Thousand-headed borecole. *B. oleracea* L. var. *ramosa* (DC.) Alefeld.

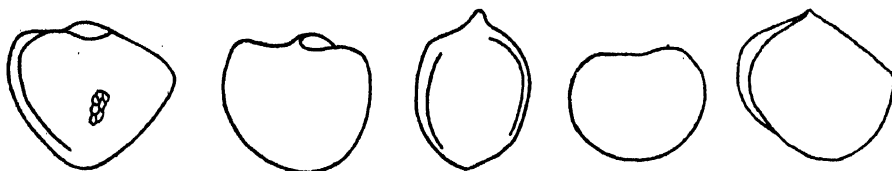
Collards. *B. oleracea* L. var. *acephala* DC.

White-ribbed winter borecole. *B. oleracea* L. var. *acephala* DC.

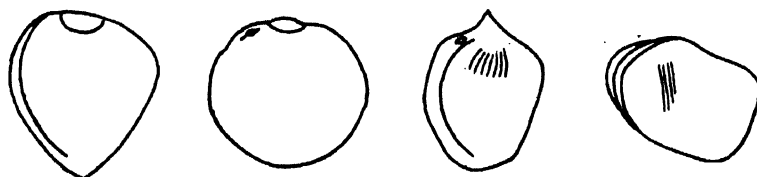
## KOHL RABI



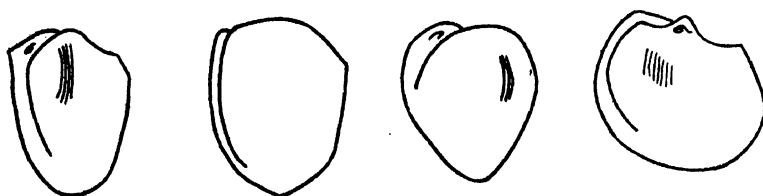
## CABBAGE



## SAVOY CABBAGE



## BROCCOLI



## CAULIFLOWER



FIG. 19. Lateral view of the following varieties and strains of *Brassica oleracea* illustrating representative shapes on which distinction of seeds may be based.

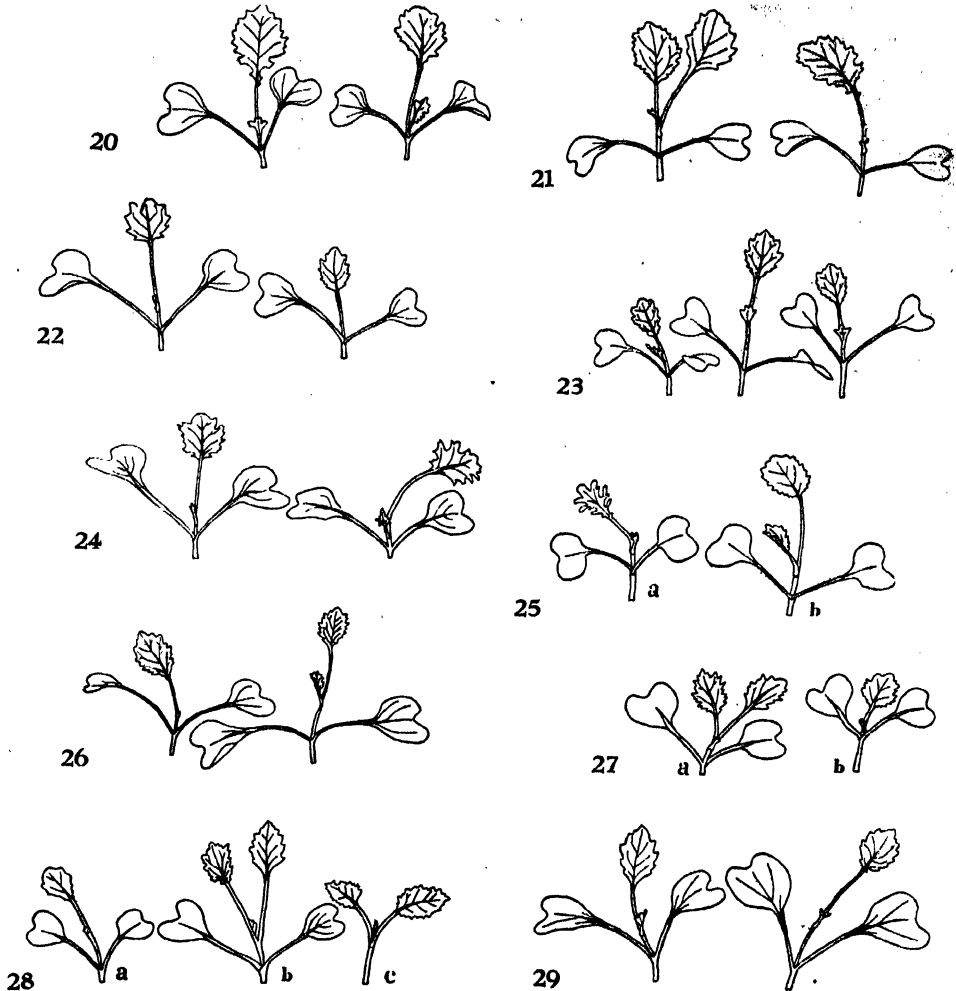
Kohlrabi. *B. oleracea* L. var. *gongylodes* L.

Cabbage. *B. oleracea* L. var. *capitata* L.

Savoy cabbage. *B. oleracea* L. var. *sabauda* (L.) Marten.

Broccoli. *B. oleracea* L. var. *botrytis* L. subvar. *asparagoides* DC.

Cauliflower. *B. oleracea* L. var. *botrytis* L. subvar. *cauliflora* (Gars.) DC.



FIGS. 20 TO 29. *Brassica* seedlings illustrating representative characters on which distinction of seedlings may be based. Drawings of photographs of growing plants.

FIG. 20. *B. balearica* Pers.

FIG. 21. *B. macrocarpa* Guss.

FIG. 22. *B. oleracea* L. var. *ramosa* (DC.) Alefeld. Flanders kale.

FIG. 23. *B. oleracea* L. var. *ramosa* (DC.) Alefeld. Thousand-headed kale.

FIG. 24. *B. oleracea* L. var. *acephala* DC. Green kale.

FIG. 25, a. *B. oleracea* L. var. *acephala* DC. Ragged Jack kale.

b. *B. oleracea* L. var. *acephala* DC. Asparagus kale.

FIG. 26. *B. oleracea* L. var. *acephala* DC. Collards.

FIG. 27, a. *B. oleracea* L. var. *acephala* DC. White ribbon kale.

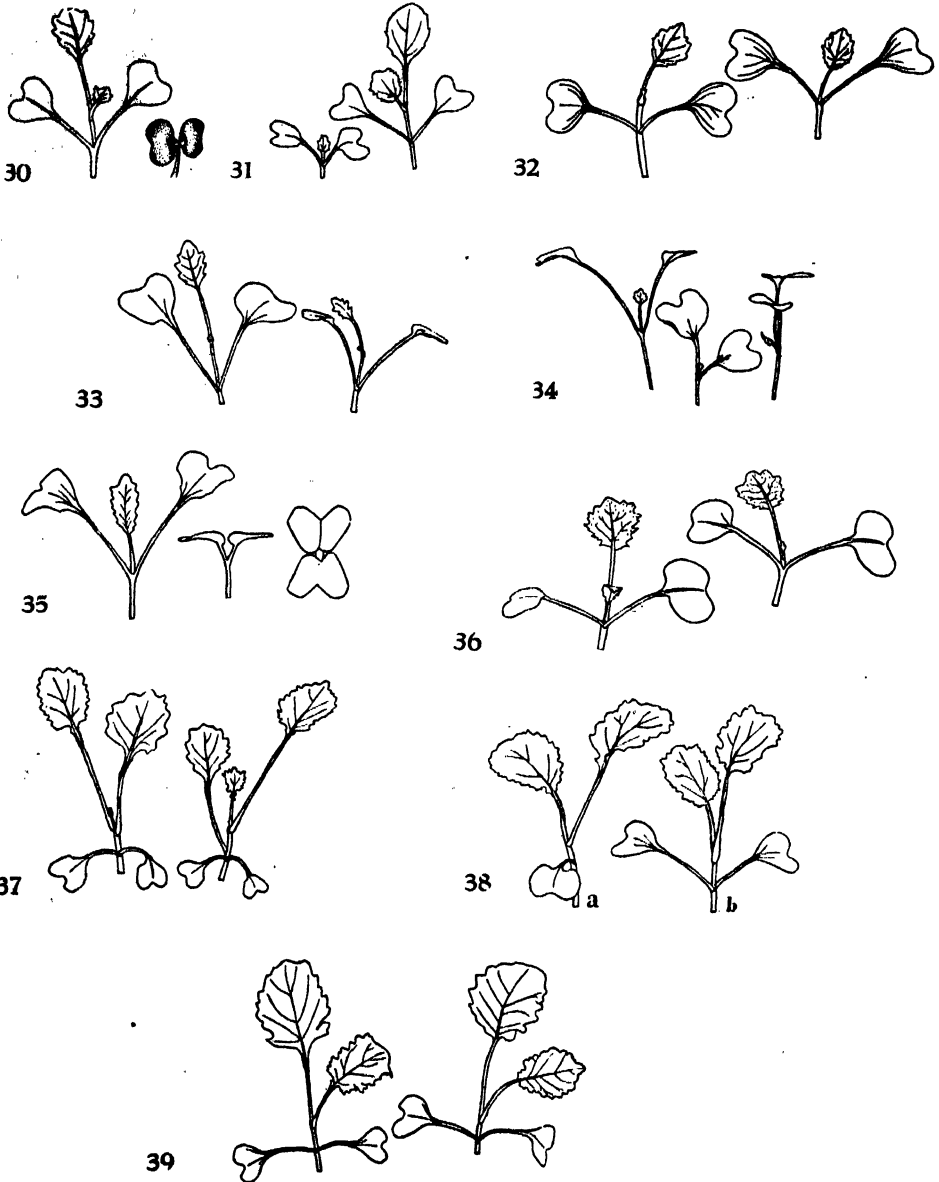
b. *B. oleracea* L. var. *acephala* DC. Cottagers kale.

FIG. 28, a. *B. oleracea* L. var. *bullata* DC. subvar. *palmifolia* DC. Palm tree kale.

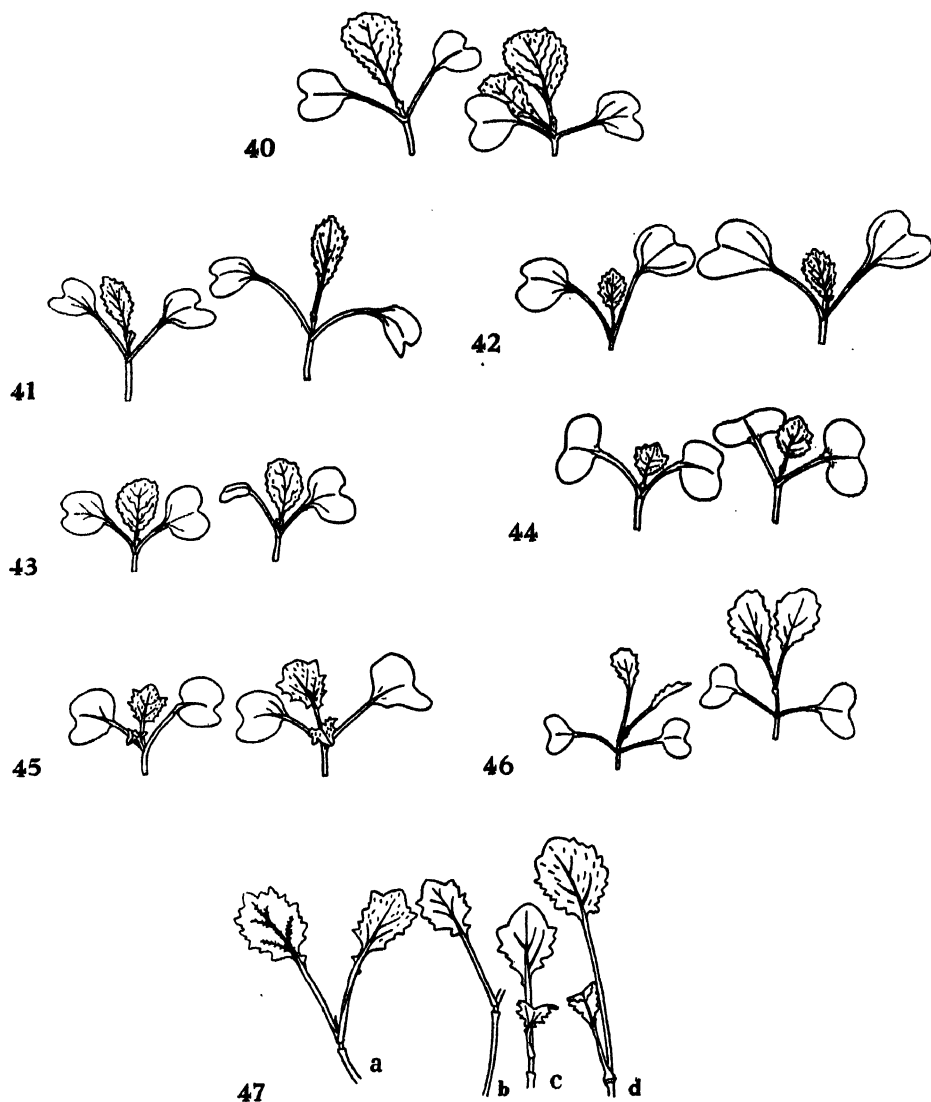
b. *B. oleracea* L. var. *acephala* DC. Milan kale.

c. *B. oleracea* L. var. *acephala* DC. Variegated kale.

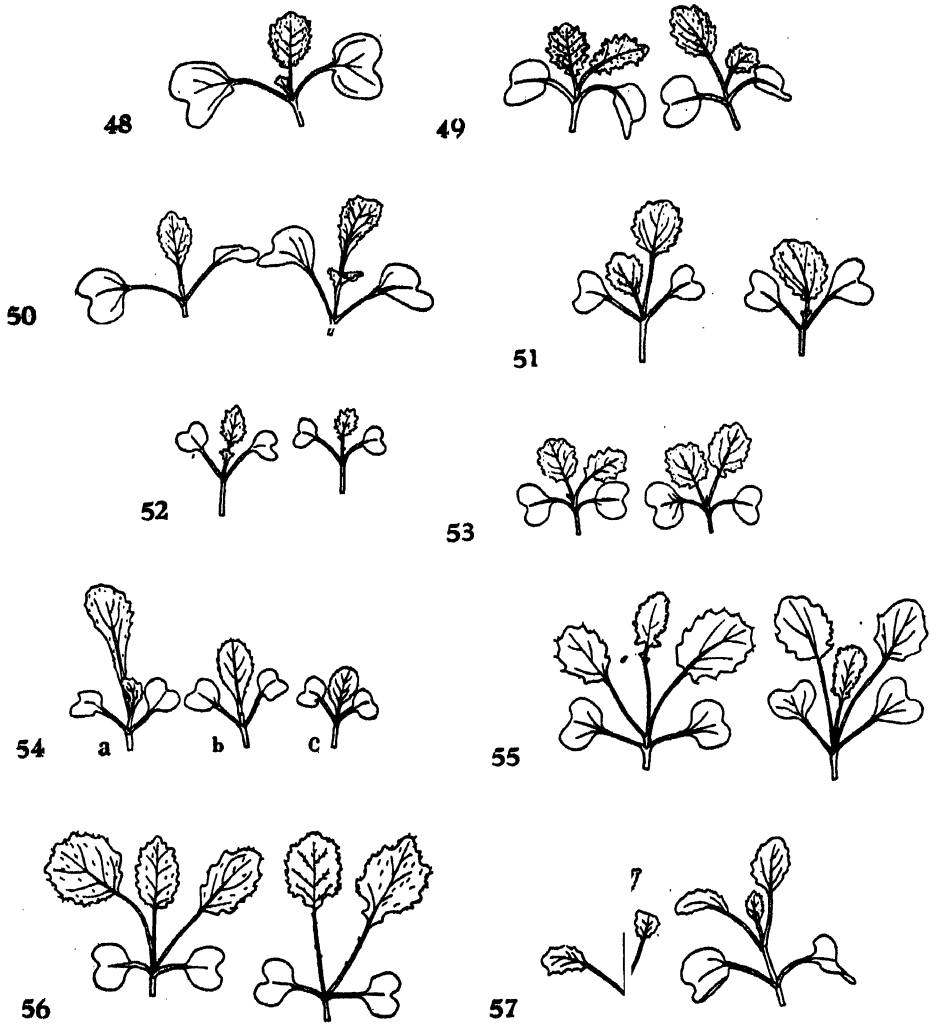
FIG. 29. *B. oleracea* L. var. *acephala* DC. Marrow kale.

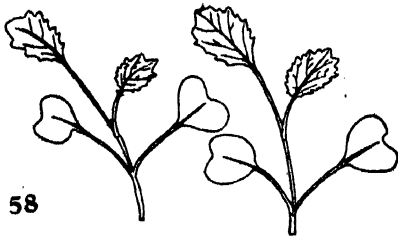


- FIG. 30. *B. oleracea* L. var. *sabauda* (L.) Mart. Savoy cabbage.  
 FIG. 31. *B. oleracea* L. var. *gemmifera* (DC.) Zenk. Brussels sprouts.  
 FIG. 32. *B. oleracea* L. var. *capitata* L. Cabbage.  
 FIG. 33. *B. oleracea* L. var. *gongylodes* L. Kohlrabi.  
 FIG. 34. *B. oleracea* L. var. *botrytis* L. subvar. *asparagoides* DC. Sprouting broccoli.  
 FIG. 35. *B. oleracea* L. var. *botrytis* L. subvar. *cauliflora* (Gars.) DC. Cauliflower.  
 FIG. 36. *B. alboglabra* Bailey. Chinese kale.  
 FIG. 37. *B. Robertiana* Gay.  
 FIG. 38, a. *B. insularis* Moris.  
 b. *B. incana* Tenore.  
 FIG. 39. *B. cretica* Lam.

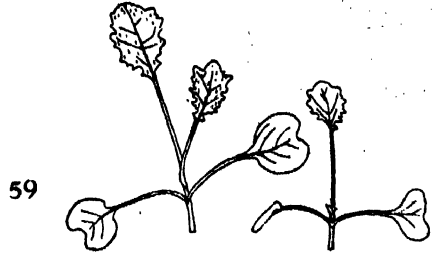


- FIG. 40. *B. pekinensis* Rupr. Wong bok.  
 FIG. 41. *B. pekinensis* Rupr. Chihili, Improved Peking.  
 FIG. 42. *B. pekinensis* Rupr. Chefoo.  
 FIG. 43. *B. pekinensis* Rupr. Market Pride.  
 FIG. 44. *B. Napus* L. Colza rape.  
 FIG. 45. *B. Napus* L. Dwarf Essex rape.  
 FIG. 46. *B. Napus* L. var. *quadrivalvis* Hook, fil Thoms.  
 FIG. 47, a. *B. Napus* L. Dwarf Essex rape.  
 47, b. *B. Napus* L. Winter rape.  
 47, c. *B. Napus* L. Colza rape.  
 47, d. *B. napobrassica* (L.) Mill. Rutabaga.

FIG. 48. *B. napobrassica* (L.) Mill. Rutabaga.FIG. 49. *B. Rapa* L. var. *rapifera* Metz. Turnip.FIG. 50. *B. Rapa* L. Shogoin turnip.FIG. 51. *B. Rapa* L. Strap leaf turnip.FIG. 52. *B. Rapa* L. var. *septiceps* Bailey. Seven-top turnip.FIG. 53. *B. campestris* L. Wild form.FIG. 54 a. *B. Rapa* L. Bailey. Shogoin turnip.54 b. *B. chinensis* (L.) Bailey. Pak choi.54 c. *B. Rapa* var. *periviridis* Bailey. Tendergreen.FIG. 55. *B. campestris* L. Biennial form.FIG. 56. *B. campestris* L. Wild form.FIG. 57. *B. campestris* L. Annual form. Bird rape.



58



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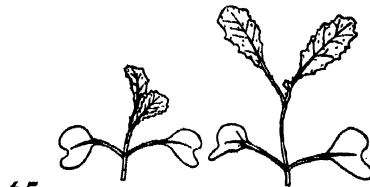
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63



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67

- FIG. 58. *B. campestris* L. Annual form. Bird rape.  
 FIG. 59. *B. campestris* L. Annual form. Bird rape.  
 FIG. 60. *B. juncea* (L.) Coss. Guy toy.  
 FIG. 61. *B. juncea* var. *crispifolia* Bailey. Curled mustard.  
 FIG. 62. *B. juncea* var. *foliosa* Bailey. Florida broadleaf.  
 FIG. 63. *B. juncea* (L.) Coss. Wild form.  
 FIG. 64. *B. integrifolia* (West) Schuls.  
 FIG. 65. *B. arvensis* (L.) Rabenh. Wild mustard.  
 FIG. 66. *B. nigra* (L.) Koch. Black mustard.  
 FIG. 67. *B. alba* (L.) Rabenh. White mustard.





SECTION C  
INDEX TO VOLUME 26

Authors

- Andreae, W. A.**—The isolation of a blue fluorescent compound, scopoletin, from Green Mountain potato tubers, infected with leaf roll virus, 31.
- Arnason, T. J., Cumming, E., and Spinks, J. W. T.**—Chromosome breakage induced by absorbed radioactive phosphorus, P<sup>32</sup>, 109.  
See Spinks, J. W. T.
- Baird, E. A. and Howatt, J. L.**—Ascorbic acid in potatoes grown in New Brunswick, 433.
- Bannan, M. W.**—Tetraploid *Taraxacum kok-saghyz*. IV. Comparison of second generation families, 115.
- Brown, A. M.**—A homothallic, short-cycle strain of *Uromyces Polygoni* (Pers.) Fuckel, 140.
- Burton, M. O., Campbell, J. J. R., and Eagles, B. A.**—The mineral requirements for pyocyanin production, 15.
- Cain, R. F. and Groves, J. W.**—Notes on seed-borne fungi. VI. *Sordaria*, 486.
- Campbell, A. B.**—See Peterson, R. F.
- Campbell, J. J. R.**—See Burton, M. O.; King, J. V.
- Clendenning, K. A.**—Growth studies of normal and parthenocarpic tomato fruits, 507.
- Cormack, M. W.**—Winter crown rot or snow mold of alfalfa, clovers, and grasses in Alberta. I. Occurrence, parasitism, and spread of the pathogen, 71.
- Cormack, R. G. H.**—A substance in the intercellular spaces of white mustard roots that reduces alkaline silver, 263.
- Corns, W. G.**—Translocation of 2,4-D in injected flowering perennial sow thistle plants, 239.
- Cumming, E.**—See Arnason, T. J.; Spinks, J. W. T.
- Currie, B. W.**—The vegetative and frost-free seasons of the Prairie Provinces and the Northwest Territories, 1.
- Eagles, B. A.**—See Burton, M. O.; King, J. V.
- Elser, H. M. and McFarlane, W. D.**—Studies on the metabolism of *Streptomyces griseus* in relation to the production of streptomycin, 164.
- Garrard, E. H.**—Fasciation of sweet peas, 158.
- Groves, J. W.**—See Cain, R. F.; Skolko, A. J.
- Hannah, A. E.**—See Peterson, R. F.
- Hedlin, R. A. and Newton, J. D.**—Some factors influencing the growth and survival of rhizobia in humus and soil cultures, 174.
- Howatt, J. L.**—See Baird, E. A.
- Hunter, L. M.**—A study of the mycelium and haustoria of the rusts of *Abies*, 219.
- Irwin, R. L. B.**—See Spinks, J. W. T.
- Jackson, H. S.**—Studies of Canadian *Thelephoraceae*. I. Some new species of *Peniophora*, 128. II. Some new species of *Corticium*, 143.

- James, N. and Smith, K. N.**—Studies on the microflora of flour, 479.
- King, J. V., Campbell, J. J. R., and Eagles, B. A.**—The mineral requirements for fluorescein production, 514.
- Landerkin, G. B. and Lochhead, A. G.**—A comparative study of the activity of fifty antibiotic actinomycetes against a variety of soil bacteria, 501.
- Langford, A. N.**—Autogenous necrosis in tomatoes immune from *Cladosporium fulvum* Cooke, 35.
- Leggatt, C. W.**—A contribution to the study of dormancy in seeds. *Lactuca sativa* L., 194.
- Loewy, A.**—See Scarth, G. W.
- McFarlane, W. D.**—See Eiser, H. M.
- McGugan, J. M.**—Seeds and seedlings of the genus *Brassica*, 520.
- Murray, B. E.**—Determining seed size by a volumetric measure, 23.
- Newton, J. D.**—See Hedlin, R. A.
- Newton, M.**—See Peturson, B.
- Nobles, M. K.**—Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi, 281.
- Peterson, R. F., Campbell, A. B., and Hannah, A. E.**—A diagrammatic scale for estimating rust intensity on leaves and stems of cereals, 496.
- Peturson, B., Newton, M., and Whiteside, A. G. O.**—Further studies on the effect of leaf rust on the yield, grade, and quality of wheat, 65.
- Richardson, E. M.**—See Trussell, P. C.
- Sanderson, M.**—An experiment to measure potential evapotranspiration, 445.
- Scarth, G. W., Loewy, A., and Shaw, M.**—Use of the infrared total absorption method for estimating the time course of photosynthesis and transpiration, 94.
- Shaw, M.**—See Scarth, G. W.
- Skolko, A. J. and Groves, J. W.**—Notes on seed-borne fungi. V. *Chaetomium* species with dichotomously branched hairs, 269.
- Smith, K. N.**—See James, N.
- Spinks, J. W. T., Cumming, E., Irwin, R. L. B., and Arnason, T. J.**—Lethal effect of absorbed radioisotopes on plants, 249.  
See Arnason, T. J.
- Strugger, S.**—Fluorescence microscope examination of bacteria in soil, 188.
- Taylor, C. B.**—Phosphorus deficiency in relation to the nitrate reduction test. Comment on a paper by Isobel Dimmick, 455.
- Trussell, P. C. and Richardson, E. M.**—Actinomycin from a new *Streptomyces*, 27.
- Tyner, L. E.**—Effect of crop debris, plant roots, and crop sequence on the microbial flora of the soil in relation to root rot in cereal crops, 86.
- Waygood, E. R.**—Adaptation of the method of Lowry and Lopez to the estimation of inorganic and organic phosphate in plant extracts, 461.
- Whiteside, A. G. O.**—See Peturson, B.

SECTION C

INDEX TO VOLUME 26

Subjects

- Abies**, Mycelium and haustoria of rusts of, 219.
- Absorption**  
of infrared, Use of, to estimate time course of photosynthesis and transpiration, 94.  
of radioactive phosphorus, Chromosome breakage induced by, 109.  
of radioisotopes, Lethal effect of, on plants, 29.
- Actinomycete(s)**, Antibiotic, Activity of, against soil bacteria, 501.
- Actinomycin** from a new Streptomyces, 27.
- Alfa**, Winter crown rot or snow mold of, in Alberta, 71.
- Antibiotic(s)**, Activity of 50 actinomycetes against soil bacteria, 501.
- Ascorbic acid** in potatoes grown in New Brunswick, 433.
- Bacteria** in soil  
Activity of antibiotic actinomycetes against, 501.  
Fluorescence microscope examination of, 188.
- Brassica**, Seeds and seedlings of, 520.
- Cereal crops**, Microbial flora of soil in relation to root rot in, as affected by crop debris, plant roots, and crop sequence, 86.
- Cereals**, Diagrammatic scale for estimating rust intensity on leaves and stems of, 496.
- Chaetomium**, Seed-borne species of, with dichotomously branched hairs, 269.
- Chromosome breakage** induced by absorbed radioactive phosphorus, 109.
- Cladosporium fulvum**, Necrosis in tomatoes immune from, 35.
- Clover(s)**, Winter crown rot or snow mold of, in Alberta, 71.
- Corticium**, New species of, 143.
- Crop debris**, Effect of, on microbial flora of soil in relation to root rot in cereal crops, 86.
- Crop sequence**, Effect of, on microbial flora of soil in relation to root rot in cereal crops, 86.
- Cultures** of wood-rotting fungi, Identification of, 281.
- 2,4-D**, Translocation of, in sow thistle, 239.
- Dimmick**, Isobel, Comment on paper by. Phosphorus deficiency in relation to nitrate reduction test, 455.
- Dormancy** in seeds of *Lactuca sativa*, 194.
- Evapotranspiration**, Potential, Experiment to measure, 445.
- Fasciation** of sweet peas, 158.
- Flour**, Microflora of, 479.
- Fluorescence microscope** examination of bacteria in soil, 188.
- Fluorescein** production, Mineral requirements for, 514.
- Forest pathology**, Identification of cultures of wood-rotting fungi, 281.
- Fruits** of tomato, Normal and parthenocarpic, Growth of, 507.
- Fungi**  
Seed-borne  
Chaetomium species with dichotomously branched hairs, 269.  
Sordaria, 486.  
Wood-rotting, Identification of cultures of, 281.
- Grade** of wheat, Effect of leaf rust on, 65.
- Grass(es)**, Winter crown rot or snow mold of, in Alberta, 71.
- Growth**  
of normal and parthenocarpic tomato fruits, 507.  
of rhizobia in humus and soil cultures, Factors influencing, 174.
- Hauatoria** of rusts of *Abies*, 219.
- Humus**, Factors influencing growth and survival of rhizobia in cultures of, 174.

- Infrared total absorption method**, Use of, to estimate time course of photosynthesis and transpiration, 94.
- Intercellular spaces** of white mustard roots, Substance in, that reduces alkaline silver, 263.
- Isolation** of scopoletin from potatoes infected with leaf roll virus, 31.
- Lactuca sativa**, Dormancy in seeds of, 194.
- Leaf roll virus**, Isolation of scopoletin from potatoes infected with, 31.
- Leaf rust**, Effect of, on yield, grade, and quality of wheat, 65.
- Leaves** of cereals, Diagrammatic scale for estimating rust intensity on, 496.
- Lethal effect** of absorbed radioisotopes on plants, 249.
- Lopez**, Adaptation of method of Lowry and, to estimation of phosphate in plant extracts, 461.
- Lowry and Lopez**, Adaptation of method of, to estimation of phosphate in plant extracts, 461.
- Metabolism** of *Streptomyces griseus* in relation to production of streptomycin, 164.
- Microbial flora** of soil in relation to root rot in cereal crops, as affected by crop debris, plant roots, and crop sequence, 86.
- Microflora** of flour, 479.
- Microscope**, Fluorescence, Examination of bacteria in soil by, 188.
- Mineral requirements**  
for fluorescein production, 514.  
for pyocyanin production, 15.
- Mold**, Snow, of alfalfa, clover, and grasses, Occurrence, parasitism and spread of, in Alberta, 71.
- Mustard**, White, Substance in intercellular spaces of roots of, that reduces alkaline silver, 263.
- Mycelium** of rusts of *Abies*, 219.
- Necrosis** in tomatoes immune from *Cladosporium fulvum*, 35.
- New Brunswick**, Ascorbic acid in potatoes grown in, 433.
- Nitrate reduction test**, Phosphorus deficiency in relation to. Comment on paper by Isobel Dimmick, 455.
- Northwest Territories**, Vegetative and frost-free seasons of, 1.
- Parthenocarp**, Growth of parthenocarpic tomato fruits, 507.
- Pathogen(s)**, Occurrence, parasitism, and spread of winter crown rot or snow mold of alfalfa, clovers, and grasses in Alberta, 71.
- Pathology**, Forest, Identification of cultures of wood-rotting fungi, 281.
- Pea(s)**, Sweet, Fasciation of, 158.
- Peniophora**, New species of, 128.
- Perennial(s)**, Translocation of 2,4- in sow thistle, 239.
- Phosphate**, Inorganic and organic, in plant extracts, Adaptation of method of Lowry and Lopez to estimation of, 461.
- Phosphorus**  
Deficiency of, in relation to nitrate reduction test. Comment on paper by Isobel Dimmick, 455.  
Radioactive, Chromosome breakage induced by absorption of, 109.
- Photosynthesis**, Use of infrared total absorption method for estimating time course of, 94.
- Plant(s)**  
Extracts of, Adaptation of method of Lowry and Lopez to estimation of phosphate in, 461.  
Lethal effect of absorbed radioisotopes on, 249.  
Roots of, their effect on microbial flora of soil in relation to root rot of cereal crops, 86.
- Potato(es)**  
Green Mountain, infected with leaf roll virus, Isolation of scopoletin from, 31.  
grown in New Brunswick, Ascorbic acid in, 433.
- Prairie Provinces**, Vegetative and frost-free seasons of, 1.
- Pyocyanin** production, Mineral requirements for, 15.
- Quality** of wheat, Effect of leaf rust on, 65.
- Radioactive phosphorus**, Chromosome breakage induced by, when absorbed, 109.
- Radioisotopes**, Absorbed, Lethal effect of, on plants, 249.





